Project Outline

Applicants

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Project Title

Validation of a novel gene expression signature discriminating limited stage from systemic follicular lymphoma as basis for risk stratification and therapeutic management

Summary

Follicular lymphoma (FL) is typically characterized by an indolent clinical course. Nevertheless, there is marked variability in the outcome of patients: while some patients die of their disease within few months, others survive more than 10 years. Therefore, one main objective in the clinical management of FL is an adequate individualized therapy concept, which is effective in the treatment of more aggressive tumors, avoiding over-treatment of patients with more indolent disease. Currently, first-line treatment decisions are by tradition based on clinical stage and the presence of symptoms. Optimizing treatment of FL is still challenging and biologically-driven risk stratification of the patients at diagnosis, e.g. by gene expression profiling, seems to be mandatory for an individualized therapeutic approach and for the decision whether FL patients, in particular with localized stage disease, are only observed (watch and wait), treated with radiotherapy or are in need of systemic therapy. Recently, we have developed novel highly-promising GE classifiers discriminating limited stage from systemic FL with prognostic impact. Before being used to develop novel treatment strategies and before entering clinical practice, a new classification tool needs to prove its generalizability beyond the patient cohort it was derived on, to adequately deal with potential overfitting to these training data. For this purpose, we aim to validate our recently published GE classifiers in an independent patient cohort. We will generate GE data and integrate clinical data from a uniquely large sample of localized-stage FL (LFL, n=250) from national (GLA, former GLSG) and international study group trials and combine these with already available data from a similarly large systemic FL trial cohort (SFL, n=274) for statistical analysis. If validation is successful, we will try to establish routinely applicable PCR-based molecular testing that allows for a robust separation of limited-stage and systemic FL and to achieve an identification of earlier progressive limited-stage FL in clinical daily-life practice to identify patients in need of intensified regimens. These molecular tools can then be integrated into novel risk-adapted treatment strategies. Our large collection of clinically annotated molecular data from limited stages FL trial cohorts will also be available for future research on the biology and pathogenesis of this rare disease.

Objectives

Deciding on the optimal treatment of FL is still challenging and biologically-driven risk stratification of the patients at diagnosis, e.g. by GE profiling, seems to be mandatory for an individualized therapeutic approach and for the decision whether LFL patients are only

observed (watch and wait), treated with RT or are in need of systemic therapy. For this purpose, we aim

i) to validate our recently published, highly-promising GE classifier (Staiger et al. 2020) in an independent patient cohort (see below a) to e) and
ii) to optimize molecular criteria that allow for a robust separation of LFL and SFL and an identification of progressive LFL in clinical daily-life practice to identify patients in need of intensified regimens (see below f) and g).

Using a large cohort of more than 500 FL samples from patients that had been verified according to clinical staging criteria as SFL (n=274) or an independent data set of LFL (n=250), we intend to answer the question, whether

a) the GE signatures that were developed can sufficiently discriminate LFL from SFL in a patient cohort independent from the one they had previously been defined in.
b) differences in clinical and molecular features between patients classified by gene expression as LFL or SFL
c) the prognostic value of the GE signatures and single genes among patients with LFL to identify progressive LFL
d) comparatively the discriminatory and prognostic capacities of the two multi-gene GE signatures (Lasso: 63 genes, elastic net: 184 genes)
e) the discriminatory capacity of single genes in comparison to the twelve genes that showed discriminatory expression in the original cohorts

To optimize GE-based stage and risk discrimination for use in clinical practice we plan to answer also the following additional questions:

f) to re-analyze the published GE data set (training cohort of Staiger et al. 2020) in order to develop a routinely applicable qRT-PCR assay with fewer genes that reproduces the published stage signatures with highest impact on the previous training cohort

In case of successful validation of the stage discriminatory signature, we plan

g) to validate the qRT-PCR assay in the independent validation cohort of LFL collected for the present project

We plan to investigate tumor specimens from the MIR study (LFL) (as well as LFL samples from Norway and Australia) and compare the results with already available data from tumor specimens from the GLSG2000 study (SFL).

In particular, we aim to isolate RNA from the patient samples of the GLA/GLSG MIR cohort (n=47). Archived formalin-fixed paraffin-embedded (FFPE) tissue blocks are already available in Stuttgart. After QC assessment, targeted gene expression measurements will be performed with the nCounter Nanostring technology.

The obtained gene expression data can be also integrated with already available copy number alteration (CNA) and whole exome sequencing (WES) data of the patient samples.

Requested Material from the GLA
Approximately 5 to 10µm sections of the collected FFPE tissue blocks to isolate RNA from MIR cohort.

Source of clinical data from the GLA/GLSG studies
GLSG2000 trial
MIR study

Cooperation Partners within the GLA
Prof. Dr. Martin Dreyling, Prof. Dr. German Ott, Prof. Dr. Klaus Herfarth, Prof. Dr. Andreas Rosenwald, Dr. Ellen Leich, Prof. Dr. Wolfram Klapper, Prof. Dr. Oliver Weigert

Literature

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