17th International Lunenburg Lymphoma Biomarker Consortium Meeting
Duin en Kruidberg Estate, Santpoort, the Netherlands
April 11-13, 2019

Minutes

Attendees: De Jong, Kimby, Salles, Kersten, Calaminici, Advani, Raemakers, Natkunam, Sehn, Gaulard, Scott, Burton, Maucort, Rosenwald, Buske, Sander, Tooze, Lenz, Stevens, Klapper, Mendeville, IJlstra, Roemer

Absent with excuse : Hiddemann,Hoster,Gribben,Hagenbeek

Thursday, April 11th, 2019

Welcome (Daphne)

Follicular Lymphoma (Wendy)
Integrated immune microenvironment and molecular studies on "stage I versus stage III/IV disease" (Research Question 1) and ''wait&see versus immediate treatment" (RQ2)

RQ1. Stage I vs stage III/IV
- Main research question: stage I: early form of FL or biologically different disease?
- Clinical data were provided by studies and registries (n=107 stage I, n=209 stage III/IV). As expected differences in FLIPI, B symptoms, LDH, Hemoglobin etc. were found.

IHC microenvironment: statistically significant but very minor differences in PD1 (lower), FOXP3 (higher), CD8 (higher) CD68 (lower) and CD163 (lower) in stage I.
Questions/suggestions:
- Correlate with proliferation marker → no Ki67 data available → could be feasible to do
- Difference in grade 1-2 or 3a st I vs st III/IV? Data should be available → will be checked
- Look at other TFH markers?
- Look at homing receptors which might be different? Not available
- Look at PD1+ cells as percentage of all CD3 cells

IHC tumor cells: results available for 307 cases (BCL2 DAKO, BCL2 SP66, BCL6, LMO2, HGAL, CD10, MUM1, MNDA)
- No major differences; BCL2: at expression level slightly more BCL2 negative cases. More SHM?
- Morphologically and phenotypically the cases seem to represent 'true' FL (original hypothesis was that stage I cohort contained MZL cases)

FISH data: half of the stage I cases are t(14;18) negative. Still 25% unknown. With the NGS data will be look at the translocation status for BCL2 and BCL6 of all cases, and also the translocation partner.

NGS data:
- NGS copy number analysis (121 vs 212 cases): significant more losses in stage III/IV on 1p, 6q, 9p, 17p, were tumor suppressor genes are located
- No significant difference between st I vs st III/IV in number of aberrations
- Significant differences in mutations eg less mutations in epigenetic genes in stage I

Questions/suggestions:

- Difference in clinical characteristics between t(14;18) + vs -?
- Pathogenesis t(14;18) negative cases unclear. Compare TMA, mutations, copy number alterations within the stage I cohort + vs -.
- FISH: break apart → some cryptic translocations may be missed, NGS translocation data will become available in the coming months (capture probes) as well as NGS hypermutation BCL2, BCL6.
- % tumor infiltration different (may be assessed by copy number analysis)? Also combination with cancer cell fraction will be possible.

RQ2. Wait&See vs immediate treatment (stage III/IV cases)

Clinical data n=92 immediate treatment vs n=63 wait and see

- FLIPI: even in the W&S cohort 20% of the patients have a high FLIPI!

IHC microenvironment: no differences

Copy number variation (n=63 w&s vs n=61 immediate treatment (nb more dropouts in IT group?): no differences

Mutations: only 2 genes significantly different (mTOR gene higher in W&S, AUTS2 higher in IT)

GEP: 23 gene profile: not predictive for need for immediate treatment (was also not designed for this question)

Dark vs light zone: not enough markers on the array to do meaningful analyses

FOXP1 expression: >10% FOXP1 expression more cases are in the IT group. Correlation FOXP1 expression with mutation analysis will be done

Questions/suggestions:

- Combine mutations and copy number variations in same pathway
- Lead time bias?
- Confounding factors (doctor and patient preferences influence start of (new) treatment); is the selection of the two cohorts robust enough?
- Do the analysis backwards (start from the molecular data and see whether there is a segregation/clustering)
- Look at time to progression in the IT patients (now TTnT is used; because most patients in the IT group were treated in trials data on progression are probably available?
- Information on transformation available? Will be checked
- Include survival data
- Include cancer cell fraction for the copy number alterations/mutations
- Check why relatively more cases in the IT group seem to have dropped out

Conclusions W&S vs IT:

With currently studied methods (TMA IHC, molecular data, GEP, FOXP1) we cannot reliably predict for which patients it is safe to defer starting treatment at time of diagnosis.

Time lines, planned publications FL:

- discussion about combining the stage I vs III/IV paper with the W&S vs IT? Will be further discussed, because on the one hand, it may dilute the message of stage I vs III/IV while on the other hand, the techniques used are the same.

Status and future of CHL and PTCL projects for LLBC (Ranjana)

- Hodgkin lymphoma: not feasible, too many key questions have been/are going to be already addressed at the intergroup level.
- T Cell lymphoma: also not feasible, because of commitment of the samples to other international consortia.

At the moment therefore not realistic to pursue HL/PTCL projects.

Friday, April 12th, 2019

Update on MYC-DLBCL study (Andreas, Delphine)

The data from this study on the significance of MYC SH/DH/TH and role of MYC translocation partner gene (IgH vs kappa/lambda) have been presented at ASH and the manuscript has been submitted to JCO (3 weeks ago).
The clinical database consists of 5117 cases; the biomarker cohort of n=2383 cases. There are no major differences at baseline for clinical vs biomarker cohort.
264 patients had MYC-R (11%). These data were produced/analyzed locally (previously shown that MYC/BCL2 FISH is a very robust and reproducible technique). MYC-kappa/lambda was done by Siebert's lab. 107 MYC-Ig, 88 MYC kappa/lambda.
DH/TH with MYC-Ig are the real poor prognosis cases, but if they survive 2 years there seems to be a plateau in the curve.
MYC-SH no impact on PFS, but slightly poorer OS (older patients? Otherwise difficult to understand differential impact on PFS and OS. Will be checked).
IHC was done with cutoff MYC >40%, BCL2>50%.

Questions/suggestions:

- No reliable data available for DSS in the database
- what about the high grade/cases that did not have DLBCL morphology (eg blastoid)/start from all DH/TH)
- Look at the HRs not only at 2 years but also in the first year?
- MYC/BCL2 expression relation to translocation data
- further (biological) data mining MYC-DLBCL (Andreas/all); MYC partner genes other than kappa/lambda

Cathy Burton: Molecular high-grade group

ReMoDL-B: cases were classified as GCB/ABC on the illymina WG-DASL platform (which is no longer available). No advantage of adding bortezomib was found in the ABC group.
GEP data for 983 FFPE samples; no difference in outcome for ABC vs GCB.
By further analysis a group of pts was identified in the GCB group which did poorly – MHG (molecular high grade) group: these cases bring down GCB survival and contribute to lack of ABC/GCB separation. This MHG group is enriched with the DH patients.
Validated on HMRN data and on the Reddy cohort.
Cross validate this with work done in Vancouver – DH signature

David Scott: DLBCL90 GEP

- Comparing DH (MYC-BCL2) with other GCB-DLBCL results in a 104 gene signature (DHITsig)
- DHITsig identified ~ 30% of GCB only half were HGBL-DH/TH
- DHITsig pos tumors had poor outcome irrespective of DH/TH status
- Proliferation based? Cell of origin? cells seem to be not light and not dark zone, so are intercycling?
- They have a distinct mutational landscape: mutations a.o. in MYC, BCL2, CREBBP, EZH2, BCL7A etc
- Was validated on RMoDL-B cases
- WGS was done on all of the cases. Some cryptic MYC and BCL2 translocations were picked up by WGS that were not picked up by FISH
- Both MHG and DHITsig identifies a GCB group which does not have this signature and has an excellent prognosis

Clinical database (Laurie/Delphine)
N=5117 patients, 75% cohort, 25% trial patients (Vancouver/Leeds registries)
- N=800 stage I, 1200 stage II (for German cases not separated)
- Definition of extranodal site may not be consistent across study groups
- Data on salvage treatment were not routinely collected
- Response data: most probably evaluated by CT, not by PET-CT

1. Validate NCCN IPI? Was created in the US and validated on the BCCA cohort.
Problems:
- LDH actual value not available – should be checked for the large groups whether it is feasible to retrieve actual values
- About 1000 cases in the LLBC cohort were included in the BCCA validation cohort used to validate the NCCN → those should be left out for the analysis
- Maybe the registries could submit additional cases for this study question
- Look at trial vs registries

2. Focus on primary refractory patients – clarify patient population, try to retrieve those cases (eg no CR at end of treatment (EOT) or progression <6, 9 or 12 months of diagnosis) Look at elderly patients (>70-75 years of age) → check the numbers.
Problems:
- For the German cases response data were not reported, but we can also approach this from the PFS data.

3. Look at relationship of PFS and OS (multistage modeling) → can be done on existing dataset

New proposals (Cathy/David)
Develop a pan- aggressive lymphoma classifier: combination of MHG/GEP pattern (HTG EdgeSeq platform). Further validate this on the LLBC cohort? Also contribute other cases eg PMBCL/MGZL?
Suggestion by Gilles: compare different platforms (HTG vs Nanostring vs RT-MLPA based)? What is the gold standard? Will be difficult because of company policies.
Share the MYC cases from LLBC?
***Action point*: a clear study question will be formulated by Cathy and David.**

Guest lecture: Next generation biomarker based trial design (Berkhof/vd Ven)
Many large RCTs in DLBCL which are or are not biomarker-based are negative (eg REMoDL-B, Phoenix, GOYA, HOVON84). This may be a reflection of heterogeneity of DLBCL (Dubois CCR 2016) → rethink the way trials are designed: what works for which patient, not what works on average.
Use Bayesian designs and/or more frequent interim analyses to avoid continuation of RCTs which are unlikely to end up positive in the end?
Use adaptive trial design (BATTLE, I-SPY2 trials). Biomarker assessment is done at start, multiple experimental treatments are incorporated and adaptive randomization is performed.
Basket trials (eg DRUP study): multiple tumor types with similar biomarker
Enrichment designs
What do you need for good biomarker-driven trials:
- identify subpopulation in need
- Limited number of predefined marker groups
- Drugs that target specific alterations
- Early primary endpoint
- Bayesian statistical methods

Saturday, April 13th, 2019

Publication and authorship guidelines (Laurie/Daphne)
The publication guidelines were circulated prior to the meeting and are confirmed unanimously with minor amendments (see attached).
Suggestions:

- include PI's of the original clinical trials in the Acknowledgment section, which should be very inclusive. For some journals it is possible to publish 'on behalf of …' a study-group, which means the article will pop up in PubMed for all participants of the study group
- Add a line that authors should comply with the ICMJE guidelines for authorships
- Include also younger persons on a per project basis

Other 'rules' for LLBC projects:

- Projects that are funded by LLBC should be subject to an annual written progress report (in addition to reporting at the annual meeting). In addition, quarterly TC meetings by the core team leading the project together with others contributing to the project (with summary of what has been done in the preceding months) should be held.
- Most of the projects are led by a pathologist and a clinician and the core team includes the statistician
- Statisticians can also be co-leads of a project
- Draft a guideline for starting a new project, which includes funding/budgets/financial issues and submit to our 2 scientific directors Daphne and Laurie with a copy to the Board of the LLBC Foundation (Gilles , Andreas and Ton) for approval.

Discussion on future plans for LLBC

- ***Clinical database:***
    - Validate NCCN-IPI: first check whether missing data (Laurie) (mostly LDH) can be collected, otherwise probably not useful, since part of the LLBC patients were already included in the original validation of the NCCN-IPI. Also maybe update the number of cases from registries to upgrade the number of cases. Inclusion criteria will be send to the different groups.
    - Explore the primary refractory cohort (Laurie, Marie José) (define which cutoffs should be used): if PD within 6 months: 612 patients, of those 325 progression; other half death as first event), 9 months 880, 12 months 1000. Probably for 40% of the cases material is already available. The patients with death as first event are mostly older patients (median 70+). For these patients it may be worthwhile to go back to the original cases and look for more cases, relapse biopsies etc.
    - Relationship between PFS and OS (Delphine, Gilles); no additional data required → create a model that estimates the transition between different states
    - Stage I/II: tease out further data from early stage cases

- ***MYC data:***
    - IHC: look at different thresholds for MYC/BCL2 positivity? Also combine this with the reproducibility of MYC/BCL2 IHC staining and scoring. Possible pitfalls: study was done on TMA. This validation study should be done with full slides, stained according to local protocols, manually scored on the slide and maybe also on scanned slide, and compare the results with the TMA results. Wolfram has a series for which IHC, FISH and nanostring are available.
      Also add the results previously obtained demonstrating robustness/reproducibility of the MYC/BCL2 FISH. This would definitely add an impactful message to the literature. Number of cases needed (20? Will be discussed with Delphine) and source of cases, number of labs (German, Nordic, Dutch, BCCA, France (Lyon/Paris), Leeds, Stanford)

will be determined and a draft will be circulated (**Wolfram will coordinate**). Use the antibody that is used at your own lab. Scoring will be done in increments of 10%. CD20 and CD3 image will be provided digitally for orientation). Labs will be informed on estimated day of arrival of the slides.

- Compare translocation data with IHC data for MYC/BCL2 (Andreas)
- Go back to the morphology (blastoid) (Maria, Philippe, Yaso); at a later stage machine learning will be involved. Draft will be circulated.
- More biologic/molecular studies (GEP (MHG vs MYC-partner gene, DHitsig), mutation analysis). **Draft will be circulated by Cathy/David.** Andreas and Delphine will send out a list of cases which will have to be checked for availability. Further mutational analysis may interfere with ongoing projects eg by BCCA.
- Look at epigenetic data/methylation profiles (Yaso). May be complicated because we do not have purified lymphoma cells. Use Ataq-seq (Bachy)

Future of LLBC

The value of working together comes from this truly multidisciplinary team, across collaborative groups (COOP groups and institutions) with an interest in clinical and translational questions in lymphoma.

Large databases available initially aiming at validation of biomarkers with different techniques, which has shifted somewhat to discovery.

Scenarios for LLBC:

- projects are planned that do not interfere with ongoing projects of individual groups
- MYC cases are kept for future validation purposes and novel projects
- Individual groups decide to provide their cases for ongoing projects outside the LLBC

To keep the projects going it is important

- **To improve communication and activities between the annual meetings (have TC once or twice in between meetings)**
- **Improve the organization of projects (team, co-leaders, quarterly progress TCs), budgets and publication rules**

Business meeting and planning for 2019/2020 (Gilles)

Members of the Board of the LLBC Foundation : Andreas, Gilles, Ton (chair)

Company support to LLBC in 2018: 240.000 Euro (8 different companies contributed)

Current bank balance: 416.000 Euro . NB. Santpoort meeting costs still need to be subtracted.

Celgene has withdrawn support to the actual meeting itself because of compliance issues. The group feels comfortable not having companies directly sponsoring/attending the meeting.

Companies will receive the LLBC Annual Reports and will be acknowledged in LLBC publications.

- Check finances with Ton
- Check current financial obligations (eg for statistician, future plans)
- New requests for funding for 3 consecutive years have been sent out by Ton in December 2018 :
- Negative responses were received from : TG Therapeutics , Servier , BMS and Epizyme
- Pending : Takeda , Seattle Genetics
- **(New) companies to be approached by (…) for financial support 2019-2021 :**
- Janssen (**Christian**),
- Novartis **(Marie Jose**),
- Pharmacyclics/Abbvie, Morphosys and Sandoz**(Gilles**),
- Karyopharm and Astrazeneca (**Laurie**)
- Genentech / Roche (**Ton**)

- **Who has entrance to :**

- Gilead /Kite ?
- Merck / USA ?
- AMGEN ?
- Pfizer ?
- Infinity ?

Please let Ton know (a.hagenbeek@amc.uva.nl) which of the above company / companies you will be approaching so that he can send you the LLBC Fundraising package (request letter + attachments) to be sent to the respective Company !

Future meetings of LLBC

A doodle will be sent out with 2 possible dates mid-April – mid-May
2020 meeting will be held in Berlin
2021 meeting will be in Lyon
2022 meeting will be in Wurzburg

April 29th , 2019