Minutes

LUNENBURG LYMPHOMA BIOMARKER CONSORTIUM 12th International Annual Meeting London, May 14-16, 2014

Attendees: Birgitta Sanders, Andreas Rosenwald, Abigail Lee, Maria Calaminici, Daphne de Jong, Randy Gascoyne, Emanuel Bachy, Gilles Salles, John Gribben, Edie Weller, Andrew Jack, Ton Hagenbeek, Wendy Stevens, Marie José Kersten (minutes), Laurie Sehn, Philippe Gaulard, Wolfram Klapper, Eva Hoster, Eva Kimby, Michael Pfreundschuh, Yaso Natkunam, Andrew Clear

Absent with notification: Ranjani Advani, John Raemaekers

Welcome by John Gribben and Ton Hagenbeek

FOLLICULAR LYMPHOMA PROJECTS (Chairs: Daphne de Jong, Laurie Sehn)

1. Early failure vs long remission

- *Clinical data* have thus far been collected for 84 patients in the early failure group and 142 patients in the long remission group (total n=226), from Barts, the GLSG and LySA (FL2000). To have a more representative sample and to increase power it would be good if also cases from the BCCA can be included. Potentially 47 EF and 31 LR pts are available.
For grade, histologic pattern and cell type a lot of data are missing. Only important point is that grade 3b's are not included.

- *IHC*: TMAs were made by Wendy in Wurzburg (early failures and long remissions are mixed in the TMAs; n=136 cases collected thus far).
The training of the computer and scoring was done at Barts (supervised by Andrew Clear, Abby and Maria). In some cases it was difficult to actually trace the follicles (less than 20%). Not all data are analyzed yet, so no data combining the clinical data with the IHC data can be shown yet.
A reproducibility (sub)study will be done on 10 cases, because there is some subjectivity in e.g. outlining the follicles. There is also some learning curve.
Summary per staining:
    - CD3, CD8, PD1: relatively easy
    - CD4: difficult stain because also macrophages are positive
    - FOXP3: scoring of the perifollicular pattern is done manually
    - P53 only strongly positive cells were counted
    - Macrophage markers (CD68, CD163): area was used instead of number of cells, because of the extensions of macrophages.

To be discussed:

- difference between core 1 and 2; what to do if one core is missing? The cores seem to be very comparable, so results from either core can be used.
- Compared to the validation study numbers of positive cells are lower. Might be interesting to rescore the validation cores?
- How to handle missing data? This will introduce bias and will influence the power.

- *Molecular analysis* (Daphne): thus far DNA has been isolated for 112 cases (GLSG and Barts). The quality of the DNA extracted from FFPE material is excellent for both labs.

An Illumina MiSeq custom Amplicon was designed; a preliminary analysis shows that known mutations e.g. in MLL2 and EZH2 are actually identified.
Discussion points:
- Should additional markers be included based on new studies on FL-FL pairs and FL-tFL pairs, e.g.:
- CARD11 (mutually exclusive with A20?)
- MLL2, MLL3, KDM6B
- FAS: early marker for transformation?
- CDKN2A/B deletion: may arise early but induce rapid transformation
- EPHA7: no follow-up papers have been published; does not seem to be a very useful marker

- Should we use other techniques because we are currently not looking at copy number alterations. Use FISH techniques, nanostring? Or do whole exome sequencing? Goes against the starting point of LLBC being a validation platform more than being a discovery platform. Also no constitutional DNA is available for any of the patients!
- Randy has data from BCCA together with the GLSG from which input can be generated on >100 genes prior to publication (will be discussed with the GLSG). NB caveat: considerable overlap between samples/patient data used both in the LLBC cohort and in these studies currently being done by the GLSG and BCCA.

Conclusion:

- CARD11 and FAS will be added to the already agreed molecular markers, and the others should be discussed in the near future pending on the possibility that Randy might share with us the results from the study of the BCCA and GLSG, He will come back to - that

- Extra samples are necessary (to improve power and to have a better geographical distribution):
  - BCCA could provide 47 samples early failure and 31 cases long remission, for which TMAs are already in place. 10 unstained sections are needed. For these cases also flow is available and all these cases have been sequenced (Laurie and Randy will check)
  - Leeds cases: Andrew, Daphne and Maria will look at the Ethics approval
  - LySA cases: Gilles will go back to LySA about the DNA; decision will follow
  - E1496: not suitable (R maintenance instead of R-chemo)

Should the IHC data and mutation analysis be in the same manuscript?

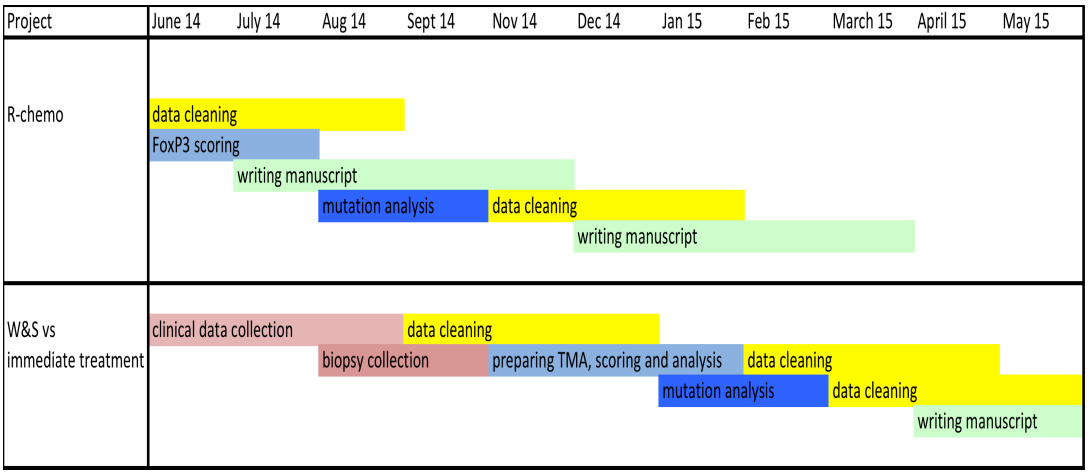
<u>W&S vs immediate treatment</u> (151 vs 488 patients with clinical data)
Table: 25 instead of 6 from Stanford; LySA cases still need to be included in the table
Discussion: should we include cases from individual centers?
John/Andrew will discuss again with Ardeshna whether w&s cases from this trial can be included (has now been published)

<u>2. Wait&See vs immediate treatment</u>

Clinical data should be available for 151 W&S patients and 488 immediate treatment patients.

<u>3. Stage I vs III/IV</u>

225 stage I patients have been identified versus 1027 stage III/IV patients.



A time line has been made for the analysis of these research questions and the writing of the manuscripts.

DIFFUSE LARGE B-CELL LYMPHOMA PROJECTS

Clinical study: Laurie/Edie presented a plan for an update of the clinical study.
Previous biomarker study: only BCL2 and Ki67 added to the IPI, and only in the low risk IPI patients. The recently published NCCN IPI (Zhou Blood 2014) appears to be better able to identify poor risk groups based on clinical markers.
Since we now have a much larger cohort of R-CHOP treated patients (4800-6000) we could on this cohort:

- validate the NCCN IPI
- Further refine this index?
- Add factors such as sex, BMI, light chains, tumor bulk, lymphocyte count, concordant BM involvement (although for some of these factors a lot of data will be missing).

A data dictionary has been made by Laurie and Edie and a request for data has already been sent to the groups.
- Include R-ABCVP (250) and MEGA-CHOEP cases? --> yes, we can then decide later whether or not to report on these cases.

FISH/IHC validation study (Andreas Rosenwald)
It is still interesting to study BCL2/MYC/BCL6, because this could provide a more definite answer on the prognostic value of these translocations and of MYC/BCL2 protein expression.
A FISH/MYC IHC validation study was performed in 6 labs (Wurzburg, Amsterdam, Vancouver, Creteil, Leeds, Stanford) with a TMA consisting of 11 cases, of which one dropped out.
- good concordance for all 3 FISH analyses (NB only 1 BCL6+ case was included in this TMA); high agreement and excellent kappa statistics
- MYC IHC: every lab was asked to stain and score (manually): this was more problematic, however with a cutoff of 40% the agreement was good (5/6 labs).
It was estimated that FISH data can be collected on 1500-2000 cases:

- BCCA: 380
- Stanford 300
- UK/Lisbon: 160
- RiCOVER: 300 + 100

- LySA: 500
- HOVON: 250
- CHOP14 trial: numbers follow
- HOVON84 trial: can become available in 2015, after the first publication

With 10% MYC+ cases and 50% failures a HR of 1.7 would reliably be picked up.
It was decided based on the validation study to:

- Accept existing FISH data (mostly MYC break apart)
- Do centralized IHC for MYC and BCL2 (Coordinated by Andreas, preferably used webbased system)
- Analyse also MYC Ig versus non-Ig partner genes (these data are also already available for the UK and LySA cases)
- Include cell of origin data? IHC data available for at least 800 cases (from Vancouver and LySA). Also gene expression data are available from a substantial number of patients from BCCA (Affy), UK (DaSL) and LySA (Affy).
- Consider pooling sequencing data in the future?

A reanalysis of the DLBCL validation study using a dichotomous analysis led to an increase in pairwise agreement between the 9 pathologists. Kappa improves to >70% only for Ki67.

Action list:
DLBCL:

- Andrew/Daphne: clear ethics approval and MTA's between LLBC and Leeds
- Laurie/Edie: check overlap with Ken Young cohort (probably non-exisistent)
- Andreas/Laurie/Edie: make timeline for DLBCL clinical study and FISH/IHC study
- LySA: check whether RCHOP and R-ACBVP cases are mixed in one TMA

All:

- check numbers of cases (with FISH data, with MYC-Ig/nonIg data)
- check Ethics approval
- input for gene list molecular analysis FL study
- respond to data request from Edie

Finances

- Additional funds are needed
- A plan will be made to approach pharma
- Approach the double hit foundation?

Next meeting: May 7-10th 2015, Boston