# Minutes LLBC meeting May 2015 Boston

Participants: Ranjana Advani, Maria Calaminici, Daphne de Jong, Philippe Gaulard, John Gribben, Ton Hagenbeek, , Eva Kimby, Sandra Lockmer, Matias Mendeville, Yaso Natkunam, Michael Pfreundschuh, John Raemaekers, Andreas Rosenwald, Gilles Salles, Birgitta Sander (minutes), Laurie Sehn, Wendy Stevens, Edie Weller, Robert Red

Celgene: Marianna Shafarenko, Kenishi Takeshita

Support: Erica Feick

Absent with notification: Marie José Kersten, Randy Gascoyne, Andrew Jack, Wolfram Klapper, Wolfgang Hiddeman

**SCIENTIFIC MEETING**

**Welcome** to Boston and the LLBC meeting by Edie Weller. Introduction of the participants.

**FOLLICULAR LYMPHOMA PROJECTS** (chaired by Daphne de Jong, John Raemaekers)

Accomplishment: The validation study of IHC markers in FL has been published. Now 3 large scale projects are ongoing in accordance with the goals and plans outlined at earlier meetings.

1. Early failure vs long remission after R-Chemo +/- R-maintenance (Wendy/Edie)

Inclusion criteria: *Early failure* defined as no remission or progression or lymphoma related death within 2 years from start of treatment with/without maintenance (R/IFN). *Long remission* defined as CR/PR more than 5 years from start of treatment with/without maintenance (R/IFN).

Number of cases collected: Cases were submitted from Barths, GLSG and LYSA. *Early failure* clinical data + biopsy n=50, included in TMA n=49, cases with all markers scored n=41. *Long remission* clinical data + biopsy n=76, included in TMA n=73, cases will all markers scored n=64.
Originally 186 cases in early failure and 196 in long remission were identified, however only about 1/3 could finally be included in the TMAs, e.g. because of no availability of blocks, no remaining material left in the block, too small biopsy samples. This is all in line with expectations. We do not have information on characteristics of dropped-out cases.

Preliminary results clinical and IHC (Wendy/Edie): Of the *clinical variables* there were no differences in patient characteristics from the different institutions. Only FLIPI score was significant between the groups. *IHC markers*: The TMA contained 2 cores of 1 mm. At least 50% of tissue should be representative in order to include the IHC score. 1 good core was enough for inclusion. Some cases were lost in scoring mainly due to too little material left in the TMA. Of the IHC markers, macrophages (CD68 and CD163) were scored as positively stained area/vs whole area and the other markers (CD3, CD4, CD8, FOXP3 and PD1 on T-cells and P53 on tumor cells), were scored as positive cells/all cells. CD20 and CD21 were used in order to define tumor areas. There were no differences in scored cases and failed cores/institution. No single marker was worse than others. Further analysis aimed to evaluate statistical correlation between markers and between markers and FLIPI to identify an optimal cutoff. The "microenvironment activity" was defined as evaluating the expression levels of 5 markers (CD3, CD4, CD8, CD163 and CD68) and defining high, low and intermediate activity based on all 5 markers and the 75$^{th}$ percentile expression level. Further analysis used percentage of positive cells, count of total positive cells/total area, % positive cells adjusted for CD3 and other correlations between populations, FOXP3 perifollicular pattern evaluated by 3 pathologists, and analysis of interfollicular, intrafollicular and total cells.
A few cases had higher percentages of CD68 and CD164 and this was not due to larger cells but higher cell numbers. In general positive correlations between IHC markers were seen. The same trend was found in the early failure and the long remission cohorts. There was no significant correlation between FLIPI and single markers. The two significant markers were CD8 and CD163 that both were significantly higher in the long remission group. CD163 showed a skewed distribution with a few cases strongly influencing the results and it was discussed whether CD163 should be leveraged or not. Without leveraging for CD163 both CD163 and CD68 retained their positive association with higher OR in the long remission group. These results were similar for evaluating the whole core and the interfollicular area but not when assessing only the intrafollicular area.

FOXP3 perifollicular pattern was manually scored by 3 pathologists (Andreas, Birgitta and Daphne) and there was (as in our previous FL validation study) a moderate agreement. This FOXP3 pattern did not differ between the early failure and long remission groups.

Suggestions for further analysis from the participants: Analyze the markers in relation to single FLIPI factors. CD8 has a rather narrow distribution, especially in the early failure group and it should be checked if this has been reported also in earlier studies (for instance in studies evaluating CD8 by flow cytometry). How much overlap is there

between CD68 and CD163 expression – could possibly be investigated by using IHC double staining. Ask the case submitters to go back to their cases and check that the patients really died from progression and also check information on transformation. Analyze also the microenvironment activity using the lowest 25$^{th}$ percentile. It was also decided to approach the LYSA PRIMA study for possible validation of the findings, likely limited to CD8 and CD163 data based on existing scores by LYSA.

The outline of the publication was discussed. Wendy has already started to write the paper.

Preliminary results of the molecular studies (Matias/Daphne): The aim is to analyze mutations associated with FL in respect to occurrence in the early failure vs long remission cohort, correlations with outcome and possible interaction with the tumor microenvironment. Mutations in KMTD2/MLL2, CREBBP, MEF2B, EZH2, EP300, FAS, TNFRSF14, CARD11, TNFAIP (A20) and MYD88 have been analyzed. BCL2 remains to be analyzed. KRAS is used as a negative control. In a technical comparison sequence capture followed by NGS on Illumina HiSeq was found to be superior to PCR-based Amplicon sequencing.
127 FFPE cores were submitted. 9 failed sequence library preparation, 5 failed due to insufficient reads (<40 x mean target coverage) and 6 lacked adequate material (no primary tumor, ASCT), thus 107 samples were left for analysis of which 44 was early failure cases and 63 belonged to the long remission group.
The average mutation burden was 2,9/sample and similar between the early failure and long remission groups. The frequency of mutations in the selected genes was similar to previously published studies with highest frequency of KMTD2 (approximately 70%) and CREBBP (around 60%). EZH2 mutations were seen in 18% of our cases. In the early failure group there was a higher number of cases with CREBBP mutations while EZH2 mutations were more frequent in the long remission group. A few patients had no mutations.
Further plans include to validate the findings some of the findings by additional PCR analysis, to analyze genome wide copy number profiles, to correlate the findings with the results of the IHC on tumor microenvironment and to study clonal evolution by analyzing the variant allele frequency and the subclonal complexity.

Suggestions from the participants were: To analyze the data in more detail before including more genes. More genes should be included based on literature review. There was also a discussion about "molecular FLIPI" in which a group of 7 genes out of 70 analyzed added discriminative power to FLIPI. It was also suggested that the LLBC group should be updated every 3 months with the gradual progression of the project.

2. Wait and see vs immediate treatment (Wendy)

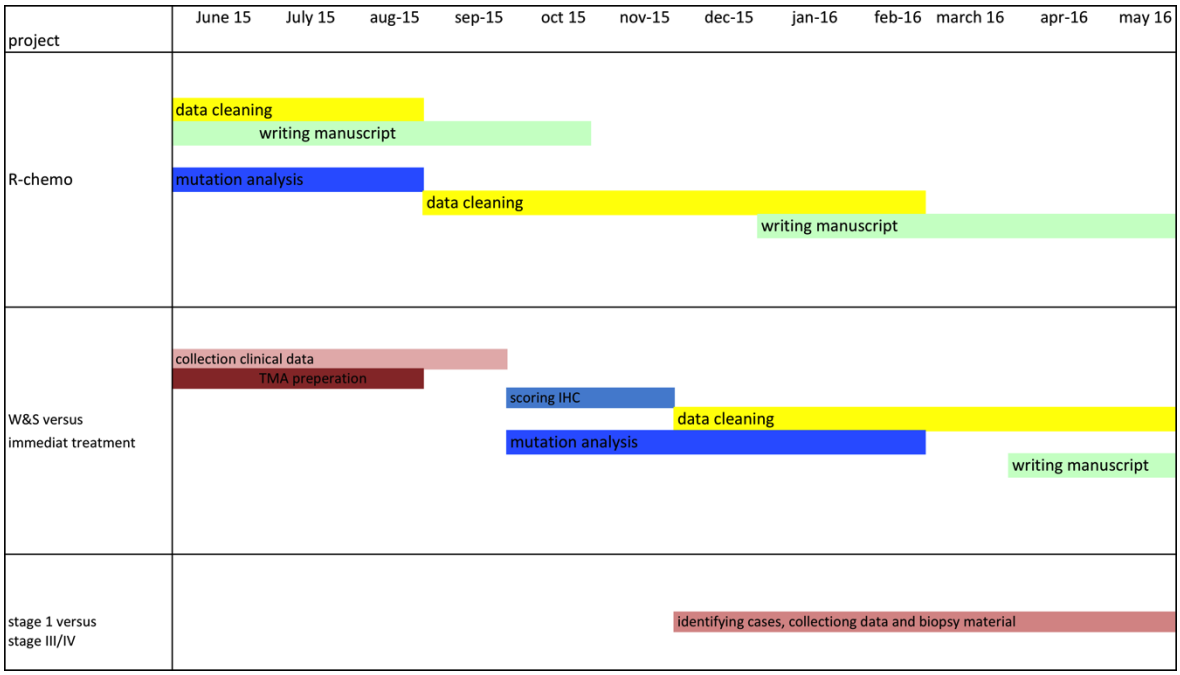
Inclusion criteria: *W&S* stage III/IV and > 5 years no treatment. *Immediate treatment* stage III/IV, < 3months after diagnosis with at least 2 of the following criteria: high LDH, tumor mass >7cm, B-symptoms, Hb<10g/L.
Cases have been collected from Sweden, Stanford, GLSG and Leeds. For clinical data 151 cases are in the W&S group and 488 in the immediate treatment group. In the TMA there are hitherto 72 patients in W&S and 113 on immediate treatments.

The numbers of included cases are lower than originally planned. It was discussed whether the inclusion criteria for the W&S arm should be changed in order to recruit more cases. It was however considered important to keep this group as clean as possible and the criteria (stage III/IV and > 5 years of no treatment) were kept unchanged. Another way to increase the number of cases would be to search registers for additional patients. Laurie will revisit whether cases from BCCA can be included. Netherlands, Norway, Barths should also be checked for more cases. Eva will contact Norway in this issue. It was also discussed which markers that should be investigated by IHC and it was decided that potentially novel markers should be identified by searching the literature, especially GEP studies on T cell/macrophage profiles in FL.

3. Stage I vs III/IV at presentation

Inclusion criteria: *Stage I* nodal disease, non-bulky < 7 cm. *Stage III/IV* >5 nodal areas, both sides of diaphragm, with/without bulky disease.
Cases have been collected from BCCA, GLSG, LYSA, Leeds and EORTC. In the Stage I group there are 225 patients with clinical data and in the Stage III/IV group 1027 patients with clinical data. It is not yet known how much tissue is available.

Time frames for the 3 ongoing studies of FL – end of spectrum:




**DIFFUSE LARGE B CELL LYMPHOMA PROJECTS** (chaired by Laurie)

Background: There is a need for better prognostic tools in DLBCL. Only 60% of patients with DLBCL are cured. Some patients have primary refractory disease and a very dismal outcome. The IPI was created before R was introduced but still there are not any new biological markers that can make the IPI redundant. However, the IPI cannot really pick out patients with poor outcome. Our previous LLBC study of DLBCL confirmed the prognostic power of IPI. In this study BCL2 and KI67 could better discriminate low risk IPI patients only. It should be noted that the R-CHOP cohort was limited (n=347) in this

study. The enhanced IPI (NCCN IPI) as presented by Zhou et al in Blood 2014 was discussed, noting that it still is not widely used.
Additional candidate clinical prognostic factors recently published were reviewed. The difficulty in translating gene expression-defined ABC/GCB profiles to IHC was mentioned. A large amount of clinical data has already been sent to Edie, and we will begin analyzing this shortly to assess prognostic significance of clinical parameters received as well as IPI and enhanced IPI.

Comments on this from the participants were: The relevance of double hits by FISH in younger patients for outcome remains uncertain. The discrepancy in reports could be due to a selection bias for performance of the FISH analyses in earlier studies.

Aims: The goal of LLBC is to investigate the prognostic significance of MYC/BCL2/BCL6 rearrangements and MYC/BCL2 expression in larger DLBCL cohorts. Ideally, already existing data from large cohorts of the LLBC members could be used.

Progress so far (Andreas):

Inclusions: Clinical data from Barths, BCCA, GHGSG, Leeds and Stanford have collected more than 5000 patients. LYSA plans to include 500 cases, which will result in approximately 6000 patients in total. FISH and IHC data is presently available from 560 cases and more will be included so the total might be 1500-2000. It is planned to include existing FISH and IHC data from Vancouver, Stanford and LYSA.

Work plan: *FISH:* the MYC-translocated cases need to be investigated for Ig/non-Ig partner including kappa/lambda which will be a large effort on approximately 150 cases. The plan is to first investigate for MYC break and if positive go to fusion probe with heavy chain. If this turns out negative then progress to light chain break and if light chain break is positive, light chain/MYC fusion will be further investigated in collaboration with Rainer Siebert. It was agreed to proceed according to this plan (in charge Andreas and Philippe).
Further cases could be found in the Hovon-84 study (Daphne, Ton and John).

*MYC/BCL2 immunohistochemistry:* Proposal to include existing data. The issue of different BCL2 antibodies was discussed and it was concluded that if findings are robust they should be detectable also in this setting and the far majority use the same antibody anyway. Very likely, different cut-offs in existing datafiles have been used (cut-offs, quartiles, 10% increment). Andreas and Philippe will investigate the various scoring protocols and available data (GLSG, LYSA, Leeds) and based on that information decide on the methods of the LLBC study to limit additional collection of data as much as possible. The timelines for the IHC cannot be decided before this issue is resolved.

Further suggestions from the participants: There was a question whether GCB/non-GCB should be included. Such information based on Nanostring is available from the BCCA and a proportion of the LYSA cases. For the LYSA cases also the Hans classifier is done. This issue will be included in the inventory questions by Andreas and Philippe.

Timelines: Partially depends on publication of primary data from some of the included cohorts. A clean dataset on FISH-results is planned to the end of 2015. Timelines for IHC

cannot yet be decided before the info on scoring cut-offs on large data-sets is available (mainly the Leeds cases).

It was further pointed out that this study will be unique in defining the importance of BCL6 translocations in DLBCL.

**Discussion considering novel projects for LLBC to bring forward**

*End-of-spectrum in DLBCL - non-responders vs cured.* This will emerge through the already ongoing study and it will be important to see how many in the category non-responders that are not due to double hit events. This group will be important to investigate for additional hits/aberrations. It was suggested to identify a group of primary progressive patients. A small group led by Edie , Laurie and Ton will organize this.

*Hodgkin lymphoma.* Andreas Engert should be invited to the next LLBC meeting to discuss this. Ton will discuss this with him.

*FL grade 3B.* How many cases with clinical data and tissue can be retrieved? (Michael and Andreas make an inventory).

**Action list follicular lymphoma**

For FL early failure vs long remission it will be investigated if cases from the PRIMA study could be used for validation of CD8 and CD163 data (Daphne/Wendy/Edie will write the proposal to LYSA).

For FL early failure vs long remission the persons who submitted cases will be contacted by Wendy to confirm that early failures were due to lymphoma related events and check on transformation status.

For FL W&S vs Immediate treatment registers will be search for additional cases; BCCA (Laurie), Netherlands (Daphne/Ton/John), Norway (Eva will contact Norway) and Barths (John and Maria).

Proposal for additional markers predicting behavior of FL: IHC and molecular analysis check markers published regarding IHC, GEP and gene aberrations (all should give input).

It was also suggested that updates from the different project should be sent out to the whole LLBC group every three months (thus starting from end of August 2015).

**Action list DLBCL**

To include further cases to the FISH project from HOVON-84 (Daphne)

To investigate the possibility to use already existing IHC stainings/scorings for DLBCL instead of making new stainings/scorings (Andreas, Philippe).

**Action list new projects**

Invite persons with interest in Hodgkin research to the next LLBC meeting (Ton will discuss this with Andreas Engert)