Minutes LLBC meeting Mery-sur-Oise, April 20-22, 2016

Participants: Ranjana Advani, Maria Calaminici, Daphne de Jong, Philippe Gaulard, John Gribben, Ton Hagenbeek, , Eva Kimby, Yaso Natkunam, , John Raemaekers, Andreas Rosenwald, Gilles Salles, Birgitta Sander, Laurie Sehn, Wendy Stevens, Delphine Maucort Boulch , Eva Hoster, Edie Weller, Andreas Engert, Luc Xerri, Thieryy Molina, Marie José Kersten (minutes)
Celgene: Myron Czuczman

Support: Carole Forot

Absent with notification: Andrew Jack, Wolfram Klapper, Wolfgang Hiddeman, Michael Pfreundschuh

Welcome by Philippe Gaulard, especially to Delphine Maucort Boulch as the new LLBC statistician.

I. Hodgkin lymphoma

Yaso Natkunam
20-30% of HL patients do not benefit from standard therapy --> Unmet need in HL: need for biomarkers for risk stratification and improved efficacy of targeted agents (either tumor cell-driven or microenvironment-driven).

Current prognostic factors in cHL:
- Factors originating from the tumor microenvironment:
- Unfavorable: genes expressed by T-cells, macrophages, pDC
- Favorable: adhesion, extracellular matrix, remodeling, B cell, fibroblast gene products

- Factors originating from tumor cells:
- unfavorable: apoptosis, cell cycle regulation, chemoresistance, escape from immune surveillance (immunosuppressive chemokines; downregulation of MHC)
- Galectin-1 (in the microenvironment, not in the HRS cells!), correlates with CD163; high Galectin-1 correlates with B-symptoms and poor outcome
- Steidl: using GEP, primary treatment failure correlates with M2>M1 type macrophages (CD68+, CSFR1), decreased B cells and cytotoxic T cells, MMP11. Using IHC, variable results have been obtained.
- ECOG E2496 trial: automated image analysis: CD68, CD163: inferior outcome, EBER not correlated with outcome
- Greaves/Gribben: CD68+ (inferior); FOXP3, CD20 (superior)
- Other possibly prognostic markers:
- Plasma microRNAs (miR-494, miR-1973, miR-21)
- Costimulator inhibitory molecules:
- CD137&CD137ligand
- PD1&PDL1/L2: increased PD1+ T cells confer poor DSS in cHL.
- PDL1/PDL2 expression can be increased by disomy, polysomy, copy gain, amplification Out of 108 HL cases the majority had copy gain or amplification (polysomy and translocations are more rare); only 1 case no 9p24.1 alteration! -->

9p24.1 alterations/PDL1/PDL2 alterations are a defining feature of cHL and likely explain the efficacy of PD-1 blockade in cHL.
- The role of EBV: leads to constitutive activation of NfkB (through LMP1) and enhances transcriptional activity of PD-L1 and PD-L2.

Opportunities for validation of potential biomarkers within the scope of an LLBC project
LLBC member groups have access to well annotated cHL cohorts, probably enriched for high risk patients
- Possible biomarkers that can be validated: focus on FFPE based biomarkers:
  - macrophage subsets (CD68, CD163, CSFR1)
  - 9p FISH; PD1/PDL2 IHC
  - CD137/CD137L
  - EBV/EBER ISH
- Other markers to consider:
  - microRNAs
  - Galectin
  - serum TARC

Ranjani Advani: advanced cHL first line
Current prognostic factors:
Clinical:
- IPS Hasenclever: less discriminative in the era of high cure rate
- Simpler prognostic score: IPS-3: Hb, age, stage IV (Diefenbach BJH 2015): could be validated in the PET-adapted treatment era
- Interim PET (Deauville score) --> use for de-escalation or escalation of therapy

Influence of new drugs: brentuximab vedotin; immune checkpoint inhibitors
Biomarkers with potential impact on outcome (prognostic): better understanding of biology and specifically the crosstalk between tumor cells and TME necessary.

Opportunities for LLBC: LLBC and its partners may have cccess to prospective study samples and clinical data of large series (a lot of them PET-adapted);
- UK PET
- GHSG
- US intergroup
- LYSA/EORTC
- FIL
- Israeli lymphoma group
- ? Company sponsored studies (Millennium/SGN)?

Possible topics:
- Prognostic factors:
  - Validate alternate IPS in PET-adapted era
  - incorporate gene signature in IPS
  - surrogate biological IPS: ML ratio
  - genetic alterations
- Predictive markers?

Discussion: Gribben and Rosenwald: very few PD1+ T cells can actually be found in HL samples (400 German cases; 200 UK samples) and they are not in close proximity to the

HRS cells. Two clones of antibodies were used that work very well in tonsil controls. You would expect exhausted T cells, also other exhaustion markers are absent.
We could also look at relapsed cases?

Andreas Engert: MRD guided treatment of HL

Salvage chemotherapy for HL:

- HD-R2 study (Josting JCO 2012): 2xDHAP followed by BEAM/autoSCT; more chemo did not add anything in that setting. Most relapses are in the first year. Prognosis for relapse after HDT is still poor, especially in case of early relapse.
- Salvage chemotherapy regimens used: Europe: mostly DHAP, US: ICE; Italy: gemcitabine-based.
- AETHERA: phase III BV vs placebo after HDT (Moskovitz Lancet 2015): HR 0.57. median PFS 24 --> 42 months; however, no difference in OS.

Pooled analysis of risk factors for outcome following salvage (n=690 pts, GHSG, LYSA, LSA): stage IV, TTR <3 mo, ECOG >2, bulk >5 cm, no response to salvage: all have a HR of 1.5-2. (Brockelmann, submitted)

Novel treatment options:

- Brentuximab is moving to first line (eg HD21: BEACOPP esc 6x vs BrECADD 6x)
- PD1/PDL1 blockers:
    - Ansell NEJM: nivolumab: very rapid responses clinically. Treatment until progression?
    - BMS phase II study: ASCO and EHA 2016 oral presentation
    - Randomized Phase II, 2x 50 patients, early favorable: cHL stage I/II without risk factors, age 18-75: 6x anti-PD1 pembro followed by 20 Gy IS-RT vs 1x antiPD1, then 20 Gy, then 5x anti-PD1. THere are indications that RT and PD1 act synergistically
    - HD20 pilot: early unfavorable; AVD +PD1 vs PD1 followed by AVD + PD1
    - Abscopal R/R HL; anti-PD-1 failure: nivolumab, RT 20 Gy, then again nivolumab

Possible MRD approaches:

- cytokine signature plasma (Casasnovas JCO 2007 sCD30, IL6, IL1RA)
- sCD163 and sTARC (Jones CCR 2013)
- circulating tumor DNA
- Genomic representation profiles (Vandenberghe Lancet Hematol 2015)

MRD guided treatment: possible areas of interest:

- first line treatment
- relapsed disease
- r/r consolidation with BV
- alloTx
- consolidation after auto/allo-SCT
- treatment guidance in pts receiving anti-PD1

Opportunities for an LLBC project:

- HL treatment is in flux

- larger numbers of pts and validation are currently lacking for a lot of prognostic markers; current studies often report on single or only a few markers --> look at a broader range of markers
- what should be the starting point --> focus on the most relevant question
- for IHC: a validation study is important
- paired samples (diagnosis – relapse; microenvironment); does it matter whether patients were treated with ABVD/StanfordV or BEACOPP? Different tumor biology in primary vs relapse material?
- rebiopsy checkpoint inhibitor treated patients --> few data yet
- clinical variables: 'low hanging fruit'; not really the focus of LLBC. Problem might be that by the time the data are analyzed the first line treatment paradigm will have changed
- identify patients that do or do not need RT? Would biomarkers be useful to predict radiotherapy responsiveness?

in relapsed disease: look at prognosis at relapse and/or in the primary material: predict for relapse (end of spectrum analysis)
End of spectrum: cure (at least 5 yr relapse-free) vs relapse <1 year after end of treatment. NB difference between ABVD and (esc)BEACOPP.

<u>Availability of tumor material for patients with relapsed disease</u> (trials or large single institution series):

- EORTC: blocks/slides at relapse were not routinely collected
- BCCA: blocks available for LLBC? Check with Randy
- LYSA: collection on TMA and collection on full blocks; centralized trials/review
- Sweden: no centralized review
- UK: TMA Hodgkin 150-200 patients
- GHSG: 99% of primary dx material ends up in one of 6 centralized review centers; no systematic review of relapsed samples
- HD-R2 study: study coordinators Hansman, de Jong

For all the trial groups: probably no matched samples available

<u>How to proceed:</u>
Which markers: IHC-based:
- macrophages CD68, CD163
- PD1, other T cell exhaustion markers
- TARC

Other techniques:
- nanostring, FISH

<u>Working party:</u>
Andreas R, Yaso, Ranjana, John/Maria, Andreas E, Laurie, John R, Daphne,
Yaso and Ranjana will take the lead

HIV related Hodgkin: John and Maria are inviting other groups to contribute cases;
Marie José is interested to participate (AMC has large cohort of HIV infected patients).

II. Follicular lymphoma project

Immunohistochemical analysis (Wendy)
Goals of the FL project
- technical validation of immunohistochemistry (published)
- prognostic biomarkers for 3 study questions: early failure vs long remission (LR); immediate treatment vs wait&see; stage I vs stage III/IV

Early failure vs LR: immunohistochemical analysis
From all the markers that were studied only 2 significant differences were found: %CD8+ cells (LR: higher CD8); %CD163 area (LR: bigger CD163 area). In the multivariate analysis: only CD8 significant (with/without FLIPI). No difference in CD3, CD4, FoxP3, CD68, PD1 or p53 expression was found.
--> conclusion: cases with LR had denser CD8 and CD163 infiltrates (total core and interfollicular areas). Also for the cases with LR more correlations between the markers (eg T cells and macrophages) seemed to be present.
For this reason a group of 22 reactive lymph nodes was studied to look for the same type of correlations (hypothesis: LR cases more resemble reactive LN). Problem is that this was a heterogeneous group (3 FH, 3 PTGC, 16 reactive cellular changes). Better to look at reactive tonsils (best example of FH)? Still those would reflect some kind of inflammation. Or look at cases of FLIS/ISFN?
It was decided that it is probably not worth the effort to redo this with reactive tonsils. The paper was submitted to and rejected by Haematologica and has now been submitted to Clinical Cancer Research. If not accepted the article will be rewritten to include the molecular data.

Molecular data (Daphne)
- Mutation analysis was performed with hybrid capture NGS. The panel used for the LR vs EF study question misses 2 markers from the mFLIPI: ARID1A and FOXO1. It probably does not make sense to redo the whole analysis.
- 127 end of spectrum patients were included, 121 with FFPE available, 111 yielded good quality molecular data. Validation was done with ddPCR for 5 markers.
- The mutation load and frequencies found are compatible with the literature; there were no specific mutually exclusive alterations.
- Most frequent mutations: MLL2 (73%) and CREBBP.
- FL copy number profile: homozygous deletions were found e.g. of the tumor suppressor genes TNFAIP3 and CDKN2A (p16)
- Significant differences: gains of chr18 more often in early failure, also gain chr8. Of the mutations EZH2 mutations were seen more often in the LR group
- Correlation between IHC and mutations:
    - MLL2 unmated correlated with high CD8/CD163
    - CREBBP: no correlation with CD4/CD8; only significant correlation with PD1
    - TNFSF14: unmutated lower CD4 and CD8
- Edie included EZH2 mutation status in a prognostic model with CD8/CD163, which yielded a HR 6.6 for long remission in case of EZH2 mutated, CD8 and CD163 high.

Future plans:
- combine copy number analysis and mutation data; assess bi-allelic events in target genes and relation to cohort

- statistical analysis: combine molecular markers + FLIPI (added value of chr18)
- writing of manuscript: submit before summer

Wait&See vs immediate treatment: data not completely analyzed yet
W&s: stage III/IV, >5 yr no treatment (n=73 IHC results available)
Immediate treatment: high LDH, B symptoms, HB<10 g/dl (n=261 clinical data, 129 IHC)
W&S significantly higher CD8 (p=0.027)
No difference in correlations between T cells/macrophages

Next steps: intrafollicular/interfollicular subsets, data cleaning, perform molecular analysis

Discussion:
- is an independent validation of our findings necessary/possible? Seems only relevant for strongly significant findings
- do we need to study other IHC markers?
- look at the tumor cell in stead of the microenvironment?
- RESORT trial: capture based sequencing was done for >200 cases (might be at diagnosis clinically comparable to the wait&see cohort)
- no data on t(14;18) available; might be relevant for the 18q gain --> check whether it is feasible to retrieve data for t(14;18) for these cases
- Vancouver series on early failure vs long remission, paper under review (4 genes discriminative)
- re-visit the gene list JCO paper Gribben group

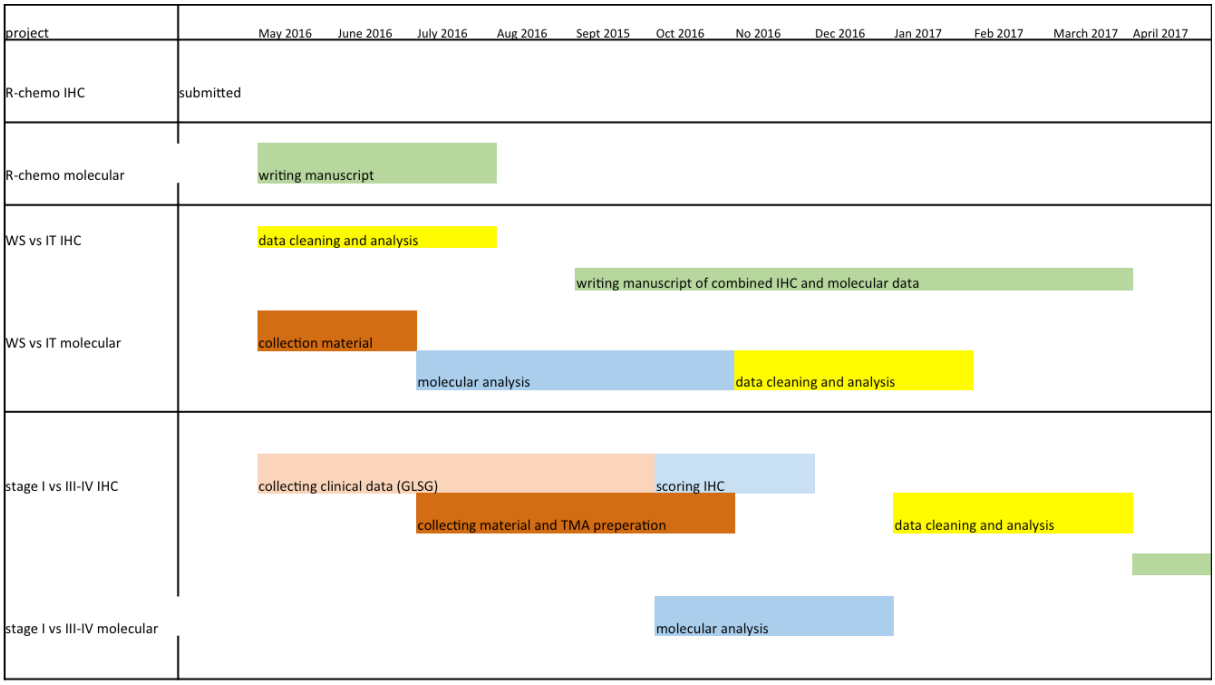
FL project, Stage I vs stage III/IV

Inventory of cases:
- Stage I: 174 cases with clinical data, probably 120-130 with biopsy material available (German cases not in TMA yet)
- Stage III/IV: 846 patients with clinical data (some have already been scored because they were also in the other cohorts (n=303))

How were the patients treated: German study IF-RT vs extended field; EORTC: IF-RT +/- TBI low dose
Approach this question a bit differently: more a biologic than a clinical question:
Hypotheses for this study question:
1. Are stage I FL biologically different from stage III/IV FL?
Are they really FL, probably some are MZL --> review all the stage I cases! Suggestion to do a path review just prior to the next meeting
Some in situ follicular neoplasia may be present and some cases with partial involvement (no ISFN in the German cases)
2. Is there a difference between stage I cases who do relapse and who do not relapse?
Questions are
- are the cases all worked up the same: the older studies will definitely not have included a PET scan, but most will have have had a bone marrow examination
- which markers:
    - t(14;18), BCL6 FISH should definitely be done
    - do we need whole sections? FISH can be done on TMA, but for review whole slides are necessary

- mutation analysis: definitely include KMT2D, CREBBP, EZH2, TNFSF14,
- copy number alterations
- should we study the microenvironment in as much detail as in the other series? Do we expect a role for the microenvironment in these cases? --> Proposal to do only CD3, CD8, CD68, CD163, BCL2, BCL6, CD10, CD20
- Trace follicles? No

TImelines FL project



Statistical analysis: how to proceed?
Edie will keep her account with DFCI and can aid transmission of the data. Robert Redd can still contribute. All the files have been transferred to Delphine.
Scheduled conference calls with Delphine, Robert and Edie. Edie and Robert will help transition of the projects. Team in Lyon is a large biostatical team consisting of 20 persons, who usually work in couples, one MD and one engineer.

How to proceed with the FL project, beyond the already defined questions?
- unique series
- potential for validation of other novel findings (also for DLBCL)
- committee should review the research question
- BCCA: series of early progressors (R-CVP treated patients studied with capseq): 4 genes important (p53 is one of them, MLL2, BTG1, XBOX protein: enriched in early progressors).

III. DLBCL project

Laurie Sehn, Andreas Rosenwald, Edie Weller
LLBC studies in DLBCL so far:

- successful study of IHC markers: BCL2, BCL6, CD5, CD10, MUM1, Ki67, HLA-DR
- BCL2 and Ki67 could better discriminate only in low risk IPI patients
- RCHOP cohort was limited in size (n=347)
- Prognostic power of the IPI was confirmed
- None of the selected biomarkers could replace the IPI

Unmet need: primary refractory patients (Hitz): very poor prognosis
Possible enhancement of IPI: R-IPI (BCCA); NCCN IPI (age 3 strata)
Other clinical prognostic factors: tumor bulk, low ALC, immunoblastic morphology, male gender, BM, sFLC, BMI (+), low lymphocyte/monocyte ratio
BIological:
- COO
- MYC/BCL2 DH translocation: will be a separate group in the new WHO classification

Current LLBC goals for DLBCL:
- Provide definitive answers on the prognostic significance of MYC/BCL2/BCL6 rearrangements and MYC/BCL2 protein expression in a very large LLBC cohort
- FISH/MYC protein validation study was done to see whether the construction of new TMAs could be skipped --> existing data from LLBC groups can indeed be used.
- Look at MYC partner genes (Ig vs non-Ig)

>200-300 MYC translocated cases appear to be available and >100 DH cases!

Edie Weller: database
- >5000 cases already in the clinical database
- Most trials are biased against DH patients (don't make it into clinical trials)
- Probably >1500 scored cases are available
    - LYSA: 03 trials, 01-5B: 800 cases in stead of 500 scored
    - BCCA 350 (scored); possibly more cases will be added
    - HOVON: 200 cases will be added

Discussion technical aspects:
- Which FISH probes were used? MYC FISH probes do matter!
- Numoz-Marmol (Histopath 63:418): Significant proportion of the non-Ig MYC translocations are outside the probe design for DAKO: half of the non-Ig partner MYC translocations will be missed with DAKO but would be picked up by Vysis probes.
- Visys: more technical failures --> actually doing both would be complementary. Vysis also misses a few centromeric translocations.
- Do Lymph2CX data on all the cases for COO analysis? 10 micron scroll would be necessary; costs around 8000 Canadian dollars
- MYC/BCL2 protein needs to be done (score in 10% increments (% positive tumor cells), also in the control group (translocation negative)
- IHC for Hans classifier will be available for all cases; Lymph2CX will be available for a large subset

Contactpersons
- LYSA: Christine Copie-Bergman FISH, Thierry Molina for IHC

- HOVON: Daphne, Marie José
- Stanford: Yaso
- Barts: Maria IHC, FISH Maria/Andreas
- Leeds: John Goodlad (successor Andrew Jack): invite him also as an LLBC member. FISH for Ig/non-Ig can be done in Wurzburg if necessary
- Vancouver: FISH, IHC done, Randy

Timelines for the paper:
- Aim for a first paper on translocation/expression data, use existing Lymph2CX and IHC data for COO
- finish FISH
- MYC break-apart still needs to be done (French samples)
- End of october all the data should be in → Delphine: data cleaning, analysis
- Paper should be submitted before the next meeting

Future plans DLBCL:
- End of spectrum analysis (early failure vs late failure, primary refractory cases)
- Subset analysis
- validate interesting results from the individual groups
- caveat: for FL separate TMAs and cores were made, for DLBCL this effort was not done!

For your agenda :

NB. Next LLBC Annual Meeting : again in Mery-sur-Oise starting on Thursday April 20 at noon and adjourning on Saturday April 22 at lunch time (and not - as originally planned - from April 27-29 !).