LUNENBURG LYMPHOMA BIOMARKER CONSORTIUM
15th International Annual Meeting
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Participants:
Thierry Molina, Philippe Gaulard, Luc Xerri, Eva Hoster, Delphine Maucort, Carole Langlois, Mad-Helene Elsensohn, Andreas Rosenwald, Laurie Sehn, Christiane Copie-Bergman, Maria Calaminici, Cathy Burton, John Goodlad, Gilles Salles, Michael Pfreundschuh, John Raemaekers, Daphne de Jong, Yaso Natkunam, Wendy Stevens, Marie José Kersten (minutes), Birgitta Sander, Eva Kimby, Ranjana Advani, Anita Ghande (Celgene), Sante Cundari (Celgene), Michael Fuchs

Absent: John Gribben, Ton Hagenbeek, Wolfgang Hiddemann , Wolfram Klapper

Welcome by Philippe Gaulard

DIFFUSE LARGE B-CELL LYMPHOMA PROJECTS
Chairs: Ranjana Advani, Philippe Gaulard

Background, study design, outcome (Delphine)
Goal of the current meeting is to define the analysis plan for the MYC project and the clinical data. Aim of the study: to assess both clinical and biologic parameters that may have prognostic impact in patients with DLBCL treated with RCHOP.
In total 5632 RCHOP treated cases were collected from cohorts (Barts, BCCA, Leeds, Stanford) and trials (German, HOVON, LYSA trials).
- Differences in clinical characteristics between cohort and trial were studied (4459 cohort; 1173 trial). The median age is higher for cohort patients (67.8 vs 65.5 years); sex distribution is similar; more pts have a PS>1. The IPI score could be calculated for 87% of patients.
- Outcome: the median FU (not provided) is longer for the cohort patients; events were censored at 120 months.
- OS: no missing data. Median OS 45 months (43 for cohort and 50 for trial); this seems low and will be checked (table does not seem to match the KM curves). No data on disease specific survival available. IPI: good stratification, but still results for cohort are worse than for trial in the different IPI strata. Outcome for patients from the Leeds cohort seems worse (however, 1/3 of patients seem to have never received RCHOP --> this will be checked)
- PFS: data still missing for Leeds and BCCA; will be collected.

Queries that need to be addressed:

- are all patients HIV negative?
- Did the patients from Leeds actually get RCHOP (how many cycles)?
- All de novo DLBCL?

Results of IHC and FISH data collection (Andreas)
In the FISH validation study there was a high rate of concordance for MYC-split FISH between the LLBC labs. Data on amplification/polysomy: more difficult on paraffin-embedded tissue; may be not available for all cases. FISH was done on TMA for Barts, Stanford, HOVON, GLSG, LYSA and on whole section for Leeds There is a risk of bias because small biopsies/needle biopsies are less likely to have been FISH-ed.

- MYC FISH data collected so far:
Cohort: 1554 non-MYC-R vs 219 MYC-R cases (14%)
Trial: 720 non-MYC-R vs 103 MYC-R which is higher than expected, one of the causes being HOVON thusfar only having submitted the MYC-R+ cases (n=24; 250 non-MYC-R still need to be submitted)

How should the data be analyzed:
- look at entire cohort but also at trial and population based cohorts separately.
- WHO update: can we assume that all submitted cases are DLBCL by morphology? In French and German trials all cases were reviewed; only a few cases could be classified as BCL-U or Burkitt-like. It is not feasible to go back and ask morphological questions for the whole group or for the MYC-IG vs MYC-non-IG (was already done by Yaso and Maria for their cases, no clear distinctive morphology)
- COO : Hans available for most cases ; Lymph2X for a considerable number of cases (German cases, BCCA).
- MYC-IG vs MYC-non-IG (MYC-non-Ig appears to have a similar prognosis to non-MYC-R; same is true for DH MYC-non-IG
- MYC/BCL2 double expressors

Impact of FISH probe (Christiane)
For LLBC, all the groups used the Vysis probe. The LYSA cases were done first by Dako and then by Vysis probe.
Dako vs Vysis probe: Munoz-Marmol Histopathology 2013
MYC breaks are highly variable and dispersed on the gene, depending on the partner gene.
Discrepancies:
DAKO+/Vysis – n= 5: all were MYC-non-Ig
DAKO +/Vysis + n= 45
DAKO-/Vysis + n=11: 7/11 DH/TH; 5/7 MYC-non-Ig
DAKO+/Vysis NI n=1
Conclusion: no probe is able to cover all MYC breakpoints; far 5' and 3' breaks may be missed. Recently a new probe was presented by Balague at EAHP 2016.
OS and MYC-R : no impact of probe
Discussion points:
What is the risk of bias in the LLBC cohort as to availability of FISH results?
- pts with very aggressive disease may have had only needle biopsies and thus no FISH
Build a new TMA/collect DNA/RNA for the MYC-R cases ?

Timelines DLBCL paper
June 2017 FISH completed ; sept 2017 analysis completed; nov 2017 paper written
Authorship and target journal to be discussed

FOLLICULAR LYMPHOMA PROJECTS
Chairs: Laurie Sehn, John Raemaekers; speaker: Wendy Stevens

FL project Q1: Early failure (n=39) vs long remission (>5 yr; n=57): published online in April in Haematologica!
Difference in FLIPI at baseline (as expected)
IHC: only statistically significant differences in CD8 and CD163 (low in EF), however very small and therefore not clinically useful differences. No impact for other markers such as PD1, FOXP1.
Molecular analysis: >90% BCL2 translocation; gain chr18 poor response
Both groups have similar mutations. EZH2 WT correlates with poor outcome (EZH2 mutations are significantly more frequent in the good outcome group)
Multivariable analysis: EZH2 and chr18 remain significant if FLIPI and IHC are added in

FL Q2 W&S stage III/IV >5 yrs (n=63) vs immediate treatment (n=100)
Immediate treatment higher FLIPI (as expected)
Median time for the W&S group to start treatment was 85 months. From the curves it seems all W&S patients received treatment, which seems unlikely?
IHC: no differences in any of the markers that were scored (on whole core, intra and interfollicular areas).
Correlation of microenvironment data with molecular data: not yet done.
On the current series the prognostic value of the FLIPI-M7 (Pastore) will be explored and some transformation/early progression markers will also be scored (eg tp53, KMT2C, BTG1, MKI67 and XBP1 (Kridel)) and POD24-PI (FLIPI + EP300, FOX01, EZH2)
Possible further analyses:
- Validate the POD24-PI on our w&s and early failure/long remission cohorts
- Do nanostring on our cohort following the results that have been generated in France (to be discussed further in Lugano (Gilles, Daphne, MJ)

FL Q3: stage I nodal disease, non bulky <7 cm (n=150); stage III/IV, >5 nodal areas (n=343)
Q3 aim of the study: is stage I a biologically different disease when compared to stage III/IV?
Few data available on the composition of the microenvironment in FL for stage I vs II-IV
Data on the stage III/IV cases have already been generated, so only the stage I cases need to be evaluated.
Andreas/Eva are currently performing similar studies on the German stage I/II vs stage III/IV cases; how this relates to the LLBC project needs to be discussed further.

Plan of analysis:
- morphology (identify 'odd' cases with FLis). Skip grading (very limited value)
- Microenvironment PD1, FOX3, CD8, CD163 (only whole core)
- add IHC markers: MUM1, H-GAL, IRTA1, MNDA, (LMO2), STATMIN
- tumor cell features by IHC (BCL2, BCL6)
- genetic alterations
    - translocation t(14;18)
    - CNV

- mutational spectrum
- 1p deletion

PERIPHERAL T-CELL LYMPHOMA

Chairs: Gilles Salles, presenters: Marie José, Philippe

Introduction

Clearly there is an unmet need for T cell lymphoma, with a worse prognosis than DLBCL (1/3 primary refractory, 1/3 relapses, 1/3 cured).

Registries: There are currently three major prospective registries: T cell project (Federico), TENOMIC (Gaulard) and COMPLETE (Hsi 2017). Analysis of the clinical data is ongoing. Thusfar no biologic studies have been performed but they are planned. T cell lymphoma is one of the subjects in the EU Harmony program.
Lymphopath project (Laurent JCO 2016): 6.3% T cell lymphoma. Problem is multiple subtypes → may be interesting to pull the data from the registries together. NB epidemiology/histology: major differences Asia vs Europe/US

Molecular profiling studies in PTCL
- GEP: TFH-derived PTCLs; new subtypes of PTCL-NOS (needs to be confirmed)
- NGS: complex landscape of somatic mutations: a few freq mutated genes and a higher number of genes mutated at low frequencies

Some are more specific for certain entities, e.g. RHOA G17V in PTCL-TFH (however also described in ATLL)
ALCL: (Gaulard Blood 2016):
- sALCL, ALK+: ALK-R and activation of STAT3
- sALCL, ALK-: IRF4/DUSP22, TP63, TK, mutations JAK1/STAT3, overexpression ERBB4)

DUSP22R ALCL: single study: subgroup with excellent outcome (Castellar Blood 2014)
CD30 is expressed by ALCL but also by many other T cell lymphomas
PTCL-NOS --> partly goes to PTCL-TFH category (IHC, flow (ICOS, cMAF, PD1, BCL6, CXCL13, CD10). Implications for therapy (hypomethylating agents)?
PTCL-follicular: very rare
AITL: 80% mutations in epigenetic regulators (TET2 (80%, IDH2, DNMT3A); 50% RHOA, 50% TCR signaling. Some of the TET2/DNMT3A occur in the hematopoietic stem cell.
Extranodal PTCL noncutaneous: NK/T, HSGDT,
All have alterations in JAK/STAT3/5 pathways
→ PTCL-NOS: which PTCL remain in this category?

Possibilities for studies in T cell NHL:
- Which biomarkers:

COO TFH, TBX21, TAT3, TCR, CD30, cytotoxic mol, CD56, EBV, HTLV1, ALK, DUSP22
- Which tools: IHC, GEP: RT-MLPA or nanostring; DNA: NGS
- Philippe has developed an RT-MLPA which can be used for classification of PTCL

Possible research questions for LLBC:
- diagnostic – epidemiology issue in view of the great variation: usefullness of molecular profiling for classification
- heterogeneity of ALK negative and ALK positive ALCL and prognostic relevance
- heterogeneity of the remaining PTCL NOS category; clinical relevance

- others: predictive markers of outcome (good vs R/R: AITL)
- small entities to be collected (indolent LPD, PTCL in immunocompromised pts)
- importanc of clinical-biological-pathological correlations
- start with easy studies molecular profiling nodal PTCL – NOS; PTCL-TFH, ALCL, AITL
- use robust molecular assays, restrict pathology review to selected (discordant) cases
- have a cohort of well-annotated PTCL pts ready to validate further biomarkers (ongoing studies)
- look at indolent PTCL cases (who never needed treatment): what is the molecular background (very rare)
- look at late relapses
- T cell proliferations arising from a reactive T cell infiltrate eg EBV related
- ALCL: several groups are looking at DUSP22; extended characterization of systemic ALCL; look at ALCL ALK+ patients with poor prognosis

Possible sources of material:
- ACT1/2: lot of needle biopsies)
- ATT (German/French)
- ECHELON-2 (75% ALCL)

HODGKIN LYMPHOMA

Chairs: Ranjana Advani, Yaso Natkunam

LLBC Hodgkin
- possible prognostic marker validation
- validation of alternative IPS in PET adapted era (simplified IPS)
- biological IPS (23 gene signature incorporated in IPS)
- surrogate biological IPS: monocyte:lymphocyte ratio

Summary of the meeting at ASH 2016:
- unclear how many cases are available in LLBC groups
- challenges: identifying uniformly treated patients (treatment will have an impact on PFS)

US: ABVD, risk adapted, Stanford V
Europe BEACOPP, ABVD
UK ABVD, risk adapted
BCC: ABVD

- currently there are no reliable biomarkers to predict prognosis in PET adapted treatment era. Ongoing studies of TARC, PD1 etc but they are not yet ready for validation by LLBC. IPS>5 is as good as known biomarkers for prediction irrespective of therapy --> is it worthwile?

Possible study design:
- end of spectrum; concentrate on failures <1 year
- finding matched controls

Specific groups:
- Engert GHSG
- Gribben Barts, Leeds, RATHL and RAPID
- Sehn BCCA and ECOG 2496
- EORTC (Raemaekers, Hagenbeek)
- LySA
- Advani Stanford

Leeds: RATHL samples have been analyzed; nanostring not significant

GHSG: HD18: samples enriched for poor prognosis are being studied with nanostring

Another possible question:
- early PET adapted treatment early stage (H10, HD16, RAPID): there is a group of patients with early negative PET scan who do relapse --> why do they relapse (matched controls 2-3:1)? Only trial patients or patients in registries with available tissue.
NB include Transplant BRaVE patients (diagnostic blocks)?

Timelines:
- 3 months for selection of cases by the groups (August 1st)

LLBC ACTION LIST

Wrap up PTCL
PTCL project action points:
A. Biomarkers predictive of outcome
- collect cases of nodal PTCL (no core needle) with the following requirements:
1. Late relapse (>5 yr) and/or long survivors without relapse vs R/R (<1 year)
Nodal any type AITL, TFH PTCL, NOS, ALCL (ALK+ and ALK-). No ATLL or extranodal TCL
2. sufficient tumor material
3. anthracyclin- containing treatment (consolidation with auto or allo is allowed)
4. diagnostic material available (if possible also relapse material)
B. Molecular classification of nodal PTCL
- characterize the heterogeneity and clinical relevance of molecular subtypes (PTCL-TFH, PTCL-NO Th1, Th2, ALK- ALCL
1. TMA locally + 1 H&E (digitalized or sent) --> extensive IHC (TFH, TBET, CD30, cytotoxic molecules, CD10, PD1, ICOS)
2. collect DNA, RNA (scrolls)
3. GEP (RT-MLPA or nanostring)
4. targeted sequencing (NGS)
Isolate DNA/RNA at the site where the tests are done (send scrolls)

Organize a meeting with Massimo Federico in Lugano to discuss collaboration (Philippe, Ranjana, MJ, Massimo, Daphne). Following the meeting in Lugano we can have a more concrete proposal and if feasible a technician or PhD student can be allocated.
Important: EU/Harmony Project is also focusing on TCL --> Gilles, Martin Dreyling and Sylvia Montoto are in the lead. This could be integrated with the LLBC project (Gilles will discuss with Martin/Sylvia). Define in/exclusion criteria and clinical database items.

Possible sources of material:
- Leeds 200 cases
- Barts
- Stanford
- Sweden registry
- TENOMICS
  T cell project
- AMC clinical database
- NL (PALGA, NKR+)
- Orphanet registries for clinical databases

- NCRI trial Royal Marsden

Wrap up DLBCL

To finish the dataset 20 FISH samples need to be done (Siebert lab) and the clinical data have to be clarified (Leeds) --> datalock in 2 months --> analysis. If HOVON can contribute the non-translocated cases (250 cases) this should be done within 2 months.
Aim for the paper: end of the year. ASH abstract? May be too early. COO will be included (Hans for all, Lymph2x for German and BCCA cases).
Additional side studies can be submitted
- e.g. compare prognostic indices, IPI vs R-IPI vs aaIPI vs NCCN-IPI
- end of spectrum analyses; sequencing of Ig vs non-Ig MYC-R.
- LMO2 as a biomarker: LMO2 negative DLBCL is less likely to have a MYC-R (misses 15% of cases).
- make a separate TMA for the MYC-R cases (will be a big effort)? Or retrieve the blocks for also in-depth molecular analyses (will be a relatively limited number of cases)
- look also at BCL2 expression alone

Wrap up FL

See summary with timelines for the 3 research questions
- (end of spectrum (published)
- w&s vs immediate treatment (analysis ongoing)
- stage I vs stage III/IV (data gathering and analysis ongoing)
Discussions were held concerning collaboration with Lysarc and with the GLSG.

Wrap up Hodgkin

August 1st: email with in/exclusion criteria (tumor material should be available for LLBC)
- End of spectrum R/R (<3 months/<1 year vs cured/late relapse)
- PET-adapted: early PET negative, no RT, relapse (EORTC, GHSG, LYSA, Stanford, Vancouver (US intergroup/BCCA)
Define biomarkers