highly sensitive method) in regard to sensitivity, specificity and efficiency of testing.
Methods. Eight different tumor cell lines, one specific for each KRAS mutation and 1 with the KRAS wild-type (wt) sequence, were used in order to perform sensitivity assay. Seven different percentages of mutated DNA in fixed amounts of wild-type DNA were tested by all the methods (10%, 1%, 0.5%, 0.1%, 0.05%, 0.01% and 0%). KRAS mutations in exons 2 (hot-spot codons 12 and 13) were evaluated by DS, ME-PCR and Pentabase Kit in sensitivity assay and in 100 consecutive CRC patients. Pentabase Kit is based on real-time PCR using SuPrimers™ (DNA primers with increased specificity), Base-Blockers™ (oligos suppressing amplification of wild-type genes) and HydrolEasy probes (hydrolysis probe with increased signal-to-noise ratio and sensitivity).
Results. In cell lines experiments, DS had a sensitivity of 10%, ME-PCR of up to 0,1% and Pentabase Kit of <0.1%. In CRC cases, we found 29% KRAS mutated patients by DS, 40% by ME-PCR and 42% by Pentabase Kit. In particular, ME-PCR detected the same mutations as direct sequencing and additional 11 cases. The Pentabase Kit found the same mutations detected by ME-PCR and additional 2 cases (G12C-G13D).
Conclusions. Overall, through the application of highly sensitive KRAS analysis methods, we detected additional KRAS alterations in up to 13/71 (18%) and 2/60 (3%) patients after DS and ME-PCR had shown them to be wild type in codons 12 and 13 of KRAS, respectively. The new kit allows the KRAS mutational typing in only 2 hours, at odds than DS and ME-PCR which require at least 2 days. Therefore Pentabase Kit represents a new, faster, more sensitive and more reliable method for the analysis of KRAS mutations in CRC, and can be proposed for the identification of patients resistant to anti-EGFR mAbs.

Corresponding author
Alice Riva, PhD Student
Institute of Pathology
Via In Selva 24
6600, Locarno, Switzerland
Phone: 0041-918160803
Fax: 0041-918160719
riva.alice@alice.it

FP47
Analysis of CD47 expression in acute myeloid leukemia in a bone marrow tissue microarray

S. Galli[1,3], C. Schuerch[1,2], I. Zlobec[1], C. Riether[2], A. Perren[1], A. Ochsenbein[2,3], Y. Banz[1,2]

[1]Institute of Pathology, University of Bern, Bern, Switzerland, [2]Department of Clinical Research, University of Bern, Bern, Switzerland, [3]Department of Oncology, University Hospital Bern, Bern, Switzerland

Background. CD47, a transmembrane glycoprotein, is widely expressed in normal and neoplastic tissues. Binding of CD47 to its receptor, signal regulatory protein-alpha (SIRPα), which is mainly expressed on macrophages, serves to inhibit phagocytosis. CD47 was shown to be up-regulated in acute myeloid leukemia (AML) and correlated with worse overall survival in patients with normal karyotype (NK) and NK-FLT3-ITD-negativity. Aim of this study was to investigate the proposed inverse correlation between CD47 and clinical outcome by immunohistochemistry (IHC) in bone marrow (BM) samples from patients diagnosed at the University Hospital Bern by establishing an AML bone marrow tissue microarray (TMA).
Methods. Of 248 newly diagnosed AML patients identified between 2006 and 2012, 184 provided sufficient high quality biopsy material for construction of a TMA from formalin-fixed, paraffin-embedded BM specimens. Protein expression by IHC was scored as low (score 0–2) versus high level staining (score 3) and correlated with standard clinical parameters and known prognostic factors in AML.
Results. CD47 staining significantly correlated with initial peripheral blast counts as well as percentage of BM infiltration (p=0.0043 resp. 0.0003). No significant correlation was found with genetic constellation or therapy mode. Unexpectedly, high CD47 expression showed a trend towards better overall (OS) and progression free survival (PFS) in the entire cohort as well as when stratified for primary versus secondary AML. In subgroup analyses, CD47 significantly inversely correlated with OS in the intermediate risk group, but no correlation was found in the favourable or unfavourable risk group or the subgroup of NK or NK-FLT3-ITD-negative patients.
Conclusions. Establishment of a BM TMA is feasible and allows for rapid analysis of CD47 protein expression by IHC. CD47 expression by IHC on BM blasts does not seem to predict worse OS and PFS in this AML cohort except for the intermediate risk subgroup. These results are in contrast to previously published data from gene expression microarrays and flow cytometry from peripheral blasts. To further investigate whether CD47 protein expression by IHC on formalin-fixed BM samples is coherent with gene expression and flow cytometry, these IHC results will be correlated with mRNA, in situ hybridisation and flow cytometric analysis of BM and peripheral blasts from patients in this test cohort.

Corresponding author
Yara Banz
Institute of Pathology
University of Bern
Murtenstrasse 31
3010 Bern, Switzerland
Phone: +41 31 632 88 75
yara.banz@pathology.unibe.ch

FP48
CD10 expression by a subset of physiological TFH cells suggests that CD10 positivity in angioimmunoblastic T-cell lymphoma (AITL) is not aberrant and reflects ontogeny

S. Hoeller[1,3], P. Amé-Thomas[2], N. Martin-Garcia[1], C. Artchounin[1], C. Copie-Bergman[1], K. Tarte[2], P. Gaulard[1]

[1]Département de Pathologie, Inserm U[955], Hôpital Henri Mondor, Créteil, France, [2]Inserm U[917], Université Rennes [1], CHU Pontchaillou, Rennes, France, [3]University Hospital Basel, Institute of Pathology, University Basel, Basel, Switzerland

Introduction. CD10 is a well-known marker of germinal center (GC) B-cells. It is also variably expressed by neoplastic T cells in angioimmunoblastic T-cell lymphoma (AITL), which is known to derive from the follicular helper T cell (TFH) subset located within the GC. However, CD10 expression is regarded as aberrant in AITL and CD10 has not yet been reported in normal TFH. We aimed to identify CD10-expressing T cells in reactive conditions and B-cell lymphomas and to characterize this population as a subset of TFH.
Methods. Fifteen reactive lymph nodes and 6 tonsils, 24 follicular lymphoma (FL), 9 marginal zone lymphoma (MZL), 5 mantle cell lymphoma (MCL) and 6 nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) were analyzed for the presence of CD10-expressing T cells by immunohistochemistry.