Acute myeloid leukemia - Biology 1

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AML WITH EVI1 REARRANGEMENTS ARE CHARACTERIZED BY FREQUENT SF3B1 AND IKZF1 MUTATIONS

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Background: Acute myeloid leukemias with *EVI1* translocations (*EVI1*-t AML) are a rare subtype of AML accounting for less than 3% of patients. The most frequent rearrangement, inv(3)(q21q26.2)/t(3;3)(q21;q26.2);*RPN1-EVI1*, is a distinct genetic entity in the WHO 2008 classification. It is characterized by multilineage dysplasia, atypical megakaryocytes with normal or high platelet counts and a poor outcome.
Aims: Co-occurring gene mutations are unknown in *EVI1*-t AML. We performed a comprehensive genome-wide analysis of mutations in *EVI1*-t leukemias using next-generation sequencing (NGS) to identify recurrent mutations that may provide key insights into the biology of this disease.
Methods: We performed transcriptome analysis of 9 adult *EVI1*-t leukemias, including 5 samples with *RPN1-EVI1* fusion and 4 with other rearrangements. Results were compared to our cohort of 143 sequenced AML with various other cytogenetic anomalies. Libraries were prepared with standard TruSeq protocols and sequencing was performed using HiSeq2000 (Illumina). Non tumoral DNA isolated from buccal swabs or saliva was used to confirm the somatic status of the identified mutations.
Results: Within a set of 35 genes frequently mutated in AML and other myeloid malignancies, *RAS* pathway mutations were the most frequent type in *EVI1*-t AML (6/9), followed by mutations in genes involved in RNA splicing (5/9 samples: 4 *SF3B1* and 1 *U2AF1*). *SF3B1* mutations were exclusive to the *RPN1-EVI1* subgroup and rarely detected (2/143) in non *EVI1*-t leukemias (*EVI1*-t *vs* non *EVI1*-t, p<0.0001). All other mutations identified in *EVI1*-t AML are shown in Figure 1. To identify novel mutations, we analyzed all genes with variants called in ≥2 *EVI1*-t specimens. After removing polymorphisms (dbSNP v.137), 20 genes were selected. Targeted sequencing of non-tumoral DNA revealed non-annotated inherited polymorphisms in 19/20 genes. One gene, *IKZF1*, contained 4 somatic mutations in 3/9 samples which were confirmed by Sanger sequencing of tumor cDNA. No *IKZF1* mutation was found in non *EVI1*-t AML (3/9 *vs* 0/143, p=0.0001). To our knowledge, recurrent *IKZF1* mutations have not been described in AML. In acute lymphoblastic leukemia, *IKZF1* alterations can result in haploinsufficiency or in the expression of a dominant negative isoform. Two samples shared an *IKZF1* N159S mutation. This mutant, located in one of the N-terminal zinc finger (DNA-binding) domain, is expected to have a dominant negative effect similar to the G158S variant previously described. The two additional mutations identified, R213X and p.N270KfsX6, are predicted to result in truncated proteins. *IKZF1* is located on chromosome 7p12.2 and monosomy 7 is the most frequent cytogenetic anomaly associated with *EVI1*-t leukemias. Two samples had a monosomy 7 and *IKZF1* expression was lower in these samples than in those without monosomy 7 (average RPKM: 14 *vs* 36, p=0.06), suggesting that monosomy 7 might result in *IKZF1* haploinsufficiency in *EVI1*-t AML. In null mice, the loss of Ikaros is associated with abnormal red cells, increased megakaryocytes and elevated platelet counts (Lopez *et al.,* PNAS, 2002), a phenotype similar to that described in *EVI1*-t leukemias.

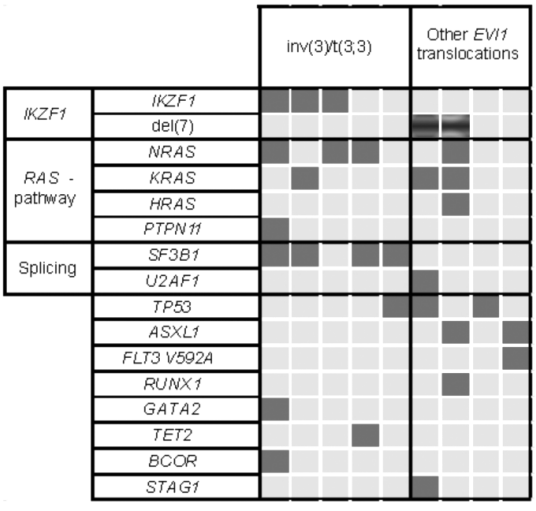

Figure 1. Mutations in *EVI1*-t AML.

Summary and Conclusions: We provide the first genomic characterization of AML with *EVI1* rearrangements by NGS. This study reveals frequent *SF3B1* and novel recurrent *IKZF1* genetic lesions. Although these results need to be confirmed in a larger cohort, we propose that *IKZF1* mutations and deletions might contribute to the distinct phenotype of these leukemias, especially in the *RPN1-EVI1* AML subgroup.

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SEQUENTIAL CARBOXY-TERMINAL PHOSPHORYLATION OF EVI1 UPON DNA DAMAGE

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Background: The *EVI1* proto-oncogene is overexpressed in 10% of acute myeloid leukaemia (AML) and is associated with very poor prognosis. *EVI1* overexpression is also a key event in leukaemic transformation in Fanconi Anaemia (FA), an inherited defect in the DNA damage response with extreme predisposition to malignancy. The *EVI1* gene encodes a DNA binding protein with roles in gene expression and epigenetic regulation. Detailed understanding of EVI1 protein regulation is incomplete, however an important role for post-translational modification is emerging. We hypothesised that protein phosphorylation might be govern EVI1 function.
Aims: Identify and characterise EVI1 phosphorylation events plus their downstream effects.
Methods: Endogenous EVI1 from EVI1-overexpressing FA-derived SB1690CB AML cells was analysed after enrichment using mass spectrometry. EVI1 protein was analysed from untreated and irradiated (X-rays; 10 Gy) cells and specific phosphorylation events identified. Phospho-specific antibodies were generated and used to confirm EVI1 phosphorylation on a specific serine residue, assess phosphorylation dynamics and characterise the effect of kinase inhibitors. Phosphorylation site-mutated EVI1 was expressed in bone marrow progenitor cells and the impact on myeloid transformation determined.
Results: In untreated cells we detected an EVI1 phosphorylation site on serine-860 (Ser860). However, after DNA damage EVI1 was phosphorylated on both Ser860 and Ser858. Induction of the doubly phosphorylated form of EVI1 was seen within 15 min of DNA damage and peaked after one hour. In contrast, the cellular pool of EVI1 phosphorylated on S860 alone was reduced, suggesting that Ser858 and 860 phosphorylation is dynamically phosphorylated in response to DNA damage. The Ser858 phosphorylation site matches the ATM kinase motif. Treatment with the ATM inhibitor KU-55933 abrogated the DNA damage-dependent phosphorylation on Ser858. Increase in the proportion of blast cells was detected in serial replating assays with phosphomimetic EVI1 mutants compared with a non-phosphorylatable EVI1, indicating that S858 and S860 phosphorylation modulates EVI1 function.
Summary and Conclusions: DNA damage modulates EVI1 phosphorylation, which may regulate its function in myeloid transformation and self-renewal.

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SETBP1 MUTATIONS IN 106 PATIENTS WITH THERAPY-RELATED MYELOID NEOPLASMS

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Background: Therapy-related myeloid neoplasms (t-MN) are myeloid disorders developing in patients treated with radiotherapy and/or chemotherapy for cancer or autoimmune diseases. t-MNs are characterized by high incidence of complex karyotypes and frequent monosomies and/or deletions of chromosomes 7 and/or 5, whereas the recently identified mutations of epigenetic regulators and of the spliceosome machinery are rare, with the exception of SRSF2. Recently, new sequencing technologies have enabled large screening of somatic mutations in myeloid malignancies, leading to the discovery of new hot spot mutations in genes candidate as leukemic transformation drivers. Among these, SET binding protein 1 (SETBP1) mutations, previously associated to Schinzel-Giedion syndrome, were reported in several myeloid neoplasms, in particular in patients with chromosome 7 alterations and SRSF2 mutations.
Aims: Object of our study was to determine the frequency of SETBP1 mutations in a cohort of 106 t-MN patients.
Methods: The study population included 106 patients affected by t-MN. According to the proportion of blasts, there were 53 t-MDS and 53 t-AML. Karyotype was abnormal in 52 of 81 (64.19%) patients in whom the karyotype was available. Chromosome 7 alterations were present in 16/81 (19.75%).
Mononuclear cells (MNCs) were separated from the BM at the time of diagnosis by Ficoll gradient centrifugation. DNA was extracted using a QIAamp DNA

Mini Kit (Qiagen). Detection of SETBP1 mutations was performed by Sanger sequencing (Life technologies). t-MN patients were also tested for mutations in DNMT3A, IDH1, IDH2, SRSF2, U2AF1 and SF3B1. Paired T-test was performed to test the association between SETBP1 mutations and other screened genes and patient's karyotype.
Results: We identified three point mutations in the SKI-homologous domain of SETBP1 in our patient cohort (3/106; 2.83%). Two patients had a G870S (COSM1234973) and one a S869R mutation. Two of three SETBP1 mutated patients (both carriers of G870S mutation) also had a SRSF2 mutation at position P95 (P95H and P95R with contextually P96 insertion). No other associations between SETBP1 mutations and spliceosome machinery or epigenetic regulators somatic mutations were found. All three SETBP1-mutated patients had a different primary tumor (Non-Hodgkin lymphoma, breast and thyroid cancer), but interestingly, all patients had developed a t-MDS (one RAEB1 and two RAEB2). None of the t-AML patients resulted mutated. Looking at karyotype associations, the two patients carriers of a G870S mutation had a complex karyotype without chromosome 7 aberration, whereas the S869R mutation carrier had a chromosome 7 deletion. The low number of SETBP1-mutated t-MN patients precluded survival analysis in this cohort.
Summary and Conclusions: We found a low incidence of mutations in the SKI-homologous domain of SETBP1. In our t-MN patients SETBP1 and SRSF2 mutations were frequently associated, whereas there was no association between SETBP1 mutation and chromosome 7 alteration, suggesting a limited role of these mutations in t-MN pathogenesis.

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FANCONI ANEMIA AND DNA-REPAIR GENE VARIANTS IN THERAPY-RELATED MYELOID NEOPLASMS

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Background: Therapy-related myeloid neoplasms (t-MN) include myelodysplastic syndromes and acute myeloid leukemias occurring as a late effect of chemotherapy and/or radiotherapy for a primary malignancy or for autoimmune diseases. The incidence of this complication has been raising in the last years due to the prolonged survival and the higher number of treated patients. Still, less than 5% of patients exposed to cytotoxic drugs and radiotherapy develop a t-MN, suggesting underlying individual susceptibility. The association of breast and other cancers to myeloid neoplasms is frequent in Fanconi Anemia (FA), a syndrome characterized by chromosomal instability, developmental abnormalities, aplastic anemia and predisposition to cancer. So far, mutations in FANC genes have been rarely described in hematological malignancies outside the syndromic picture of FA.
Aims: We were interested in the prevalence of FANC and other DNA-repair gene variants in t-MN following cytotoxic treatment for breast cancer and lymphoproliferative diseases.
Methods: The test- patient cohort included 37 patients with a t-MN. According to the proportion of blasts, there were 19 t-MDS and 18 t-AML. The primary malignancy was Hodgkin lymphoma (HL) in 7 patients, non-HL in 12 patients and breast cancer in 18 patients. DNA isolated from BM-MNCs at t-MN diagnosis was analyzed using Agilent HaloPlex system and validated with Sanger sequencing and Pyrosequencing. We selected 41 genes, including 14 FANC pathway genes and 27 further DNA repair genes. For the ATM SNV analysis, we included BM samples from further 60 t-MN patients, adding to a total of 97 t-MN patients (48 t-MDS and 49 t-AML). Control samples were obtained from 129 Caucasians with a negative history for previous malignancies and normal PB cell counts. Differences in the distribution of ATM SNV between patients and controls were evaluated using the Fisher's Exact test.
Results: DNA-repair and FANC gene variants were frequent in our t-MN patients, with 21 of 37 patients (57%) carriers of at least one genomic variant. There were no differences in latency from the primary cytotoxic treatment and t-MN in mutated *vs* unmutated patients and the median overall survival after t-MN diagnosis was similar for the two groups. The gene with the highest frequency of variant sequences was TP53 (15 variants in 10 patients). Looking at FANC genes, we found 7 heterozygous variants in 6 patients (16%), including two FANCA (L6F and S90T), three FANCD2 (T1376A, P256S and M1023V), one FANCJ (I364V) and one FANCC (L36F). Six variants were novel, according to the NCBI and Cosmic databases, whereas the FANCA L6F had been previously described (*www.1000genomes.org*). Control tissues confirmed that the variants were germ-line in 5 patients with available material (Table 1). The frequency of FANC variants in our t-MN cohort was higher than that reported in 200 *de novo* AML patients by the Cancer Genome Atlas Research Network.
In the extended study population, the ATM P1054R variant was significantly more frequent in t-MN compared to controls (10.3% *vs* 2.3%, p=0.018). This translates into a 4.8-fold increased t-MN risk.

Table 1. Characteristics of patients with FANC and DNA-repair variants.

UPN	Primary Tumor, age	t-MN latency, Karyotype	Gene Symbol and AA Change	NCBI rs and/or COSMIC Numbers	Control Tissue
6531	Breast, 57 yrs	72 months, del5q31	FANCA L6F	rs189841793	Breast cancer and normal breast tissue
3155	NHL, 69 yrs	66 months, complex	FANCA S90T	novel	NHL biopsy
5993	HL, 52 yrs, Thyroid	48 months, Del7	FANCD2 T1376A	novel	HL biopsy and normal lymphnode
6190	HL, 47 yrs	208 months, Trisomy 8	FANCD2 P256S	novel	Buccal swab and hair follicle
6828	HL, 60 yrs	112 months, n.a.	FANCD2 M1023V	novel	HL biopsy
2885	Breast/ Adrenal, na	na	FANCJ I364V FANCC L36F	novel novel	

NHL: Non-Hodgkin Lymphoma, HL: Hodgkin lymphoma.
NCBI and Cosmic database variant number and the reported frequency are indicated.

Summary and Conclusions: The high incidence of FANC germline variants in our t-MN cohort may be the underlying cause for increased susceptibility to primary cancers and may induce secondary leukemogenesis.
In the same line, the higher prevalence of the ATM P1054R SNV in t-MN may increase sensitivity of hematopoietic progenitor cells to the DNA damaging effect of chemo- and radio-therapy leading to secondary leukemia.

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SELECTION OF A PRE-EXISTING TP53 MUTATED CLONE IN THERAPY-RELATED ACUTE MYELOID LEUKEMIA

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Background: Therapy-related myeloid neoplasms (t-MNs) are thought to arise due to mutational events in hematopoietic stem and precursor cells induced by cytotoxic treatments for a primary disorder. However, no consistent biomarker has been identified yet that unambiguously classifies a particular neoplasm as "therapy-related". This raises the possibility that other mechanisms may also be operational in their pathogenesis.
Aims: Our aim was to demonstrate that leukemia-specific mutations were pre-existing in some of these patients and we, therefore, focused on the *TP53* gene which is frequently mutated in t-MNs.
Methods: We used Sanger sequencing of paired samples - t-MN and constitutional material - for initial mutation identification and rearrangement-specific PCR for the detection of a *TP53* duplication in pre-leukemic specimens, respectively. Quantitative PCR was performed to semi-quantify the mutated clone and Ion torrent deep sequencing (Life Technologies, Carlsbad, CA) to search for co-operating mutations.
Results: We screened 53 t-MN specimens for *TP53* mutations and identified one patient with a somatic heterozygous 64-base pair duplication (NM_000546.5:c.276_339dup:p.Leu114Profs*31) who developed therapy-related acute myeloid leukemia (t-AML) with complex karyotype (46-50,XY,del(5)(q12q33),?r(7)(p22q11)[cp20]) 13 years after combined modality treatment for Hodgkin's lymphoma. This duplication was particularly amenable for detection by a highly sensitive PCR assay enabling the detection of 0.01% rearranged cells. We could not only unambiguously detect the presence of *TP53* mutated cells in the patient´s pre-treatment bone marrow but also in a lymphadenitis sample obtained seven years before lymphoma diagnosis. Quantitative PCR estimated the amount of affected bone marrow cells as <1% as compared to the t-AML specimen. Analysis of the *FLT3*, *NPM1*, *ASXL*, *TET2*, *IDH1/2*, *HRAS*, *KRAS*, *RUNX1*, *MLL*, *JAK2*, *WT1*, *PTEN* and *PHF6* genes by deep sequencing using bone marrow and leukemia specimens did not show evidence of co-operating mutations.
Summary and Conclusions: The fact that a leukemia-specific *TP53* mutation is already present before any chemo- or radiotherapy has been administered suggests a different mode of therapy-related leukemogenesis than currently assumed. Instead of inducing a leukemia-specific mutation, cytotoxic treatments have facilitated the expansion of a pre-leukemic clone in this patient.

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GENOMIC ANALYSIS OF THE CLONAL ORIGIN AND EVOLUTION OF ACUTE PROMYELOCYTIC LEUKEMIA IN A UNIQUE PATIENT WITH A VERY LATE (17 YEARS) RELAPSE

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Background: Acute promyelocytic leukemia (APL) is a distinct subtype of acute

myeloid leukemia characterized by a balanced reciprocal translocation t(15;17)(q22;q12-21). The introduction of all-*trans*-retinoic acid (ATRA) has considerably improved complete remission (CR) rate and survival of APL patients. APL is now a highly curable disease and late relapses beyond 7 years are very rare. Here we describe a female patient with manifestation of APL 17 years after the initial APL diagnosis (Figure 1A).

Aims: To elucidate whether the more recent manifestation of the disease is a very late relapse or a *de novo* APL in this unique patient by genomic analyses.

Methods: Whole genome sequencing (WGS) and array-comparative genomic hybridization (CGH) were performed on genomic DNA from the patient's bone marrow specimens at initial (1994) and second diagnosis (2011), and blood samples during second CR.

Results: The patient was initially treated with ATRA and chemotherapy in 1994 while ATRA and chemotherapy plus arsenic were given in 2011 (Figure 1A). WGS revealed two different *PML/RARα* gene fusions (Chr17: 38489469-Chr15:74316176 and Chr15:74316160-Chr17:38489139) in APL cells from both samples, with the first fusion being predominant in both (Figure 1B). The fusion genes were further verified by Sanger sequencing (Figure 1B). Although the fusion genes/breakpoints were identical, significant differences in genetic aberrations were observed between the first and second APL samples, as revealed by WGS and array-CGH. Importantly, WGS and the fragment length analysis demonstrated FLT3ITD and FLT3-D835 point mutation in the first and second APL samples, respectively.

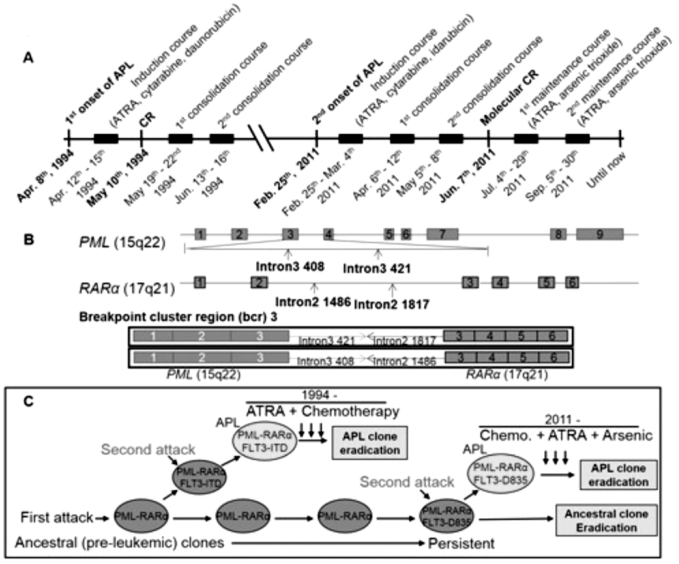

Figure 1.

Summary and Conclusions: We believe that our patient documents the longest interval between diagnosis and relapse of APL described in the literature to date. Genomic analyses show how APL clones evolved between the two manifestations of APL in this patient (Figure 1C). Likely, the patient's hematopoietic cells underwent *PML-RARα* gene fusion following a genetic attack, leading to the generation of abnormal ancestral or pre-leukemic clones, which is consistent with a recent study. These clones then transformed into APL following acquisition of *FLT3ITD* by another genetic attack. The patient obtained a CR following ATRA treatment plus chemotherapy in 1994. APL blast clones were eradicated after treatment, but the ancestral clones carrying the *PML-RARα* fusion gene were persistent and acquired a *FLT3-D835* point mutation later. The *PML-RARα/FLT3-D835* clones then contributed to the second onset of APL in 2011. At relapse, the patient was given ATRA and chemotherapy as induction and ATRA/arsenic acid as consolidation therapy. The patient has now remained in a molecular remission for three years, hopefully, indicating that a "non-aggressive" approach was appropriate for this patient. In summary, we here describe an extremely late relapse of APL and the leukemic clonal evolution. Very late relapses in APL, as seen in this unique patient, are more likely caused by a new genetic attack on existing pre-APL clones, which differs from early relapses resulting from the re-growth of original residual APL blasts. Genomic characterization of late relapses may have therapeutic implications.

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NOVEL MUTATED GENES IN ACUTE PROMYELOCYTIC LEUKEMIA IDENTIFIED BY WHOLE-EXOME SEQUENCING

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Background: Acute Promyelocytic Leukemia (APL) is a rare hematologic neoplasm characterized by the fusion gene *PML/RARA*. However, it is known that this abnormality itself is not able to reproduce the whole leukemic phenotype. Preliminary APL whole exome studies have identified a huge number of somatic mutations affecting 135 different genes in a non-recurrent manner, except for *FLT3*, *WT1* and *KRAS*. These findings suggest that APL is a heterogeneous disease with secondary relevant changes not yet defined.

Aims: We performed whole-exome sequencing (WES) of tumor-normal matched samples to identify new gene mutations that might carry prognostic value in APL. Novel candidate-genes, together with other previously described[1,2], were resequenced in an independent cohort of APL patients. The obtained results were further studied by *in silico* analysis to enlarge our understanding of their role in the pathology and entry genetic pathways.

Methods: WES was performed on matched samples from 5 *de novo* APL patients, as our study cohort. Library preparation and exome capture were performed according to the protocol version 2.1 from Baylor College of Medicine for sequencing with SOLID 4 platform as recommended by the manufacturer. WES data were analyzed using and in-house bioinformatic pipeline. Candidate variants were confirmed by Sanger sequencing. We extended the analysis of these variants to a validation APL cohort (n=76). Furthermore, a custom panel of 97 genes (17 genes from in-house results and 80 genes reported to be mutated in at least 1 patient from APL previous studies[1,2]) was performed on a subset of the validation cohort (n=25) using SureDesign Tool (Agilent) for NGS, according to the manufacturer's instructions. Samples were provided by the Hospital La Fe Biobank.

Results: We identified 32 SN*Vs* non-synonymous coding mutations and 18 small indels, with an average of 10 mutations per sample (range 7-14). We confirmed 17 SN*Vs* and 1 indel of the candidate variants (36%) in 17 genes by Sanger sequencing. Among them, no recurrent specific variants were observed through the study and the validation cohorts, with the exception of *FLT3*. Over the 25 patients analysed by the genes panel, we detected a mean of 7,76 mutations per sample (range 1-23). We identified a total of 92 variants affecting 54 different genes, where 33 were mutated in more than one patient. When we focused our research on these genes, we found that 10 mutated genes (2 of them, listed from our WES results) had a higher frequency in our cohort than expected in the 1000g database ($P \leq 0,01$). Pathways analysis of the mutated genes are been analyzed and the complete results will be presented in the meeting.

Summary and Conclusions: This study shows a comprehensive analysis of APL combining WES with the frequency assessment of somatic mutations from a custom panel of targeted genes by NGS. We identified recurrent deleterious mutations in 10 genes with a strong potential to be involved in APL pathogenesis. The relevance of the newly defined mutated genes for APL pathogenesis will require functional validation studies. *This work was supported by PI12/01047; RD12/0036/0014; RD09/0076/00021; PROMETEO/2011/025.*

References

1. Greif PA, *et al.* Leukemia. 2011 Sep;25(9):1519-22.
2. Cancer Genome Atlas Research Network. N Engl J Med 2013; 368:2059-2074.

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DNA METHYLATION PATTERNS AT DISEASE RELAPSE IN ACUTE MYELOID LEUKEMIA TARGET CONVERGENT ELEMENTS AND PATHWAYS DESPITE INTER-PATIENT HETEROGENEITY

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Background: Disease relapse is a fundamental clinical challenge for Acute Myeloid Leukemia (AML) since most patients have poor clinical outcomes. The biological basis of relapse in AML remains unclear, and a role for epigenetic mechanisms has not been examined in depth. Genetic evolution of the disease after induction treatment is widely believed to underlie the emergence of chemotherapy resistant clones. However, recent reports have identified only a limited number of somatic mutations and copy number abberations upon disease relapse, and in some cases, no relapse-specific events were detected. These findings suggest a role for other mechanisms in relapsed AML. We hypothesize that epigenetic plasticity and dysregulation of biological pathways contribute to the relapsed disease phenotype in AML.
Aims: In this study, we aimed to determine epigenetic patterning (DNA methylation) and its potential functional consequences in relapsed AML.
Results: DNA methylation profiling using Enhanced Reduced Representation Bisulfite Sequencing (ERRBS) of 39-paired diagnosis and relapse AML patient samples was performed. Cells analyzed were enriched for the blast population. We identified thousands of statistically significant changes in cytosine methylation patterns. All patients acquired both hyper- and hypomethylated differentially methylated CpGs (DMC) and regions (DMR) resulting in a range of cytosine methylation patterning upon disease relapse. In spite of the variety of cytosine methylation patterns, the majority of differentially methylated cytosines are located in intergenic regions in all cases. Interestingly, however, a subset of promoters were hypermethylated in almost all patients at relapse. This heterogeneity is not uniformly driven by mutations in epigenetic modifiers known to affect DNA methylation; has been confirmed in two independent patient cohorts (n=71 and n=31 paired diagnosis and relapse AML patient samples); and is reproducible in a xenograft model of relapsed AML treated with cytosine arabinoside (Ara-C). Integrated analysis with ENCODE datasets revealed enrichment for differential cytosine methylation upon disease relapse at distal regulatory elements (including enhancers and insulators) and regions affected by histone marks associated with cell cycle and transcriptional regulation. Pathway enrichment analysis of differentially expressed genes (determined from RNA-seq) and genes affected by differential methylation within their promoters upon disease relapse revealed convergence on commonly affected biological pathways between the patients. In particular, there was a strong overlap between gene promoters differentially methylated in relapsed compared to diagnostic AML in the patients and xenografted sample ($p<0.01$, hypergeometric test), including members of the Wnt signaling pathway.
Summary and Conclusions: We conclude that there are extensive and dynamic changes in DNA methylation patterns between diagnosis and relapse in AML. Preliminary analysis demonstrates convergent epigenetic targeting of specific gene pathways that may contribute to relapsed AML pathogenesis. The genomic distribution of reprogrammed methylation also suggests a role for epigenetic plasticity at distal regulatory elements. These findings suggest that mechanisms regulating cytosine methylation may be altered from *de novo* states after exposure to induction chemotherapy or facilitate survival of minor clones of disease during treatment resulting in widespread epigenetic reprogramming upon disease relapse.

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WHOLE EXOME SEQUENCING REVEALS CLONAL EVOLUTION PATTERNS AND DRIVER GENETIC ALTERATIONS OF RELAPSED PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background: Pediatric acute myeloid leukemia (AML) comprises ~20% of pediatric leukemia, representing one of the major therapeutic challenges in pediatric oncology. Nearly 40% of patients still suffer from a relapse after first-line therapies and once the relapse occurs, long-term survival rates decrease, ranging from 21% to 34%. The recent development of high-throughput sequencing technologies has provided an unprecedented opportunity to investigate comprehensive genetic alterations that are involved in the tumor recurrence of various cancers including adult AML. However, little is known about the molecular mechanisms of relapsed pediatric AML.
Aims: The purpose of this study is to identify the clonal evolution patterns and the major mutational events in relapsed pediatric AML.
Methods: We performed whole exome sequencing of 6 relapsed AML cases, in which diagnostic, relapsed and complete remission samples were available. Copy number alterations and structural variants including tandem duplications were also analyzed using whole exome sequencing data. Subsequently, deep sequencing of identified mutations was performed to evaluate intra-tumor heterogeneity and the clonal origin of relapsed clones.
Results: Whole exome sequencing of 18 samples from 6 patients with different subtypes of pediatric AML were analyzed with a mean coverage of more than x100, by which 95% of the targeted sequences were analyzed at more than x20 depth on average. The mean number of nonsynonymous mutations was higher at relapsed phase than at the time of diagnosis (14.0/case *vs* 10.5/case). Mutational signature was dominated by C>T transitions at both phases. Clonality assessment using variant allele frequencies of individual mutations revealed the presence of intra-tumor heterogeneity both at the diagnostic and at therelapse phases. In all 6 patients, relapsed AML evolved from one of the subclones that were present at the diagnostic phase. In all cases, relapsed AML was accompanied by many additional mutations that were absent (relapse specific mutations) or existed only with lower allele frequencies (relapse enriched mutations) in the diagnostic samples, indicating a multistep process of leukemia recurrence. Mutations that were specific to or enriched in relapsed specimens were found in several driver genes including *ASXL1*, *NRAS* and *CREBBP*, suggesting these mutations could contribute to tumor recurrence. In some cases, AML relapse may accompany a dynamic clonal change. For example, some *bona fide* driver mutations, such as *KRAS* mutations, that were predominant at the time of diagnosis disappeared in relapsed samples.
Summary and Conclusions: Whole exome sequencing unmasked the clonal structure of primary and relapsed pediatric AML, which helped to understand the underlying mechanism of relapse in pediatric AML. Our results suggested that the intra-tumor heterogeneity was common in pediatric AML both at presentation and at relapse and subclonal mutations involving RAS pathway genes and chromatin modifiers could contribute to the AML relapse.

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IDENTIFICATION OF SOMATIC MUTATIONS OR FUSIONS BY RNA-SEQUENCING IN ACUTE MYELOID LEUKEMIA.

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Background: For patients with diagnosis of acute myeloid leukemia (AML) the presence or absence of specific genetic alterations is useful for both prognosis and treatment choice (according to AML classification). However standard techniques, like cytogenetics and PCR, are able to identify only a limited number of alterations.
Aims: We tested RNA-sequencing in AML patients in order to validate this technique as a method to identify fusions and mutations in a clinical setting and obtain information about genetic variations in AML.
Methods: We collected samples from peripheral blood or bone marrow of 13 consecutive patients with new diagnosis AML of any type. RNA-sequencing data were generated using an Illumina Genome Analyzer IIx following standard library-prep protocols. Alignment to the reference GRCh37/hg19 genome was performed using BWA. Alignment data were processed using Samtools. Single nucleotide and small indels detection was performed using in-house software and applied to a predetermined list of 45 genes involved in AML pathogenesis (CEBPA, NPM1, FLT3, RUNX1, MLL, WT1, EZH2, NF1, MECOM(EVI1), KIT, H-RAS, K-RAS, TET2, IDH1, IDH2, DNMT3A, BCOR, BCORL1, NUP98, ASXL1, ABCB5, BAALC, CEP72, DIP2C, ROBO1, KLC1, TP53, IGFBP7, SETBP1, JAK2, NRAS, NOTCH1, CDKN2A, MPL, SF3B1, BRAF, PTPN11, SRSF2, IKZF1, GATA1, MYD88, ATM, CBL, PHF6, BCL2). The presence of fusions was assessed using FusionAnalyser.
Results: We identified a total of 9 fusions and 28 single nucleotide variants with a median number of 2 single nucleotide variants per patient (range 0-6). 5 patients had fusions that were previously detected with standard techniques (1 AML-ETO, 3 PML-RARA and 1 CBFB-MYH11); for all these patients known fusions were confirmed by RNA-sequencing. In 4 patients, previously unreported fusions were detected. Two of them were already known from the literature (ZMYM2-FGFR1 and MLL-MLLT10) and two were new (ETS2-ERG and WDFY3-WAS). The ZMYM2-FGFR1 is particularly interesting, because it is potentially targetable by using Ponatinib. Interestingly, we were able to identify new potential targeted therapies for 7 patients with single-nucleotide mutations: NOTCH1 D622E using NOTCH inhibitors; KIT N511K using Dasatinib; KRAS A155T using RAS inhibitors; MLL G256E, DNMT3A C599G, TET2 L1801R and EZH2 T80S using demethylating agents. Considering both new and already known mutations and fusions, 12 out of 13 patients could have received a patient tailored treatment based on these data (Table 1).
Summary and Conclusions: Our study demonstrates that RNA-sequencing leads to rapid detection of somatic mutations and fusions in AML patients. The possibility of recognizing a subset of genetic variations with potentially therapeutic value could pave the way to a genomic-centered, "personalized" therapy.

Table 1.

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IMPROVED COPY NUMBER VARIATION ESTIMATION FROM NEXT GENERATION SEQUENCING DATA REVEALS FOCAL GENETIC LESIONS SUCH AS MLL-PTD IN ACUTE MYELOID LEUKEMIA

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Background: Different next generation sequencing (NGS) platforms generate high-quality data which allows, in addition to the detection of mutations, small deletions or insertions (indels), for the estimation of copy number variations (CNVs). CN*Vs* in combination with concomitant single nucleotide variants (SNVs) or indels, possibly in the same regions, can be utilized for further delineation of the biology of acute myeloid leukemia (AML). Remarkably, AML patients have an exceptionally low number of CNVs, with a mean of 2.38 CN*Vs* per case as previously measured by DNA mapping arrays. The increased resolution of NGS derived high-density sequence data could enable the detection of novel genetic lesions in AML, which could serve as guides towards the identification of novel cancer-related genes.
Aims: The development of an algorithm that mitigates systematic bias from the NGS data in conjunction with the robust detection of novel genetic lesions in AML.
Methods: Whole genome sequencing was performed on 3 primary AML patients, harboring the chromosomal rearrangements inv(3)(q21q26) or t(3;3)(q21;q26), and matched *in vitro* cultured T-cells using the Complete Genomics platform. Whole exome sequencing was performed on 30 primary AML patients, 30 matched relapse samples, and 30 matched *in vitro* cultured T-cells controls using the Illumina HiSeq 2500 platform. The NGS data was analysed with an in-house developed package CNVsvd. In short, we count the number of fragments in consecutive 0.5 kb windows and compare them to the counts derived from a reference set of healthy individuals. Of a selected number of AML patients the CN*Vs* were also determined using Affymetrix 500K DNA mapping arrays.
Results: The whole genome sequencing data revealed an extensive number of small genetic lesions, more than 100 CN*Vs* per AML case, initially missed with the DNA mapping array. Most of these CN*Vs* are concomitantly detected in the matched controls and are therefore deemed to be germline CNVs, which in principal could still play a vital role in leukemogenesis. Importantly, the vast majority of CN*Vs* was corroborated by structural variants present in the NGS data. Surprisingly, on average 7 somatic CN*Vs* (sCNV) were detected per AML case using WGS data in conjunction with the CNVsvd package, while on average 2 sCN*Vs* per AML case were detected with the DNA mapping arrays. Most of these novel sCN*Vs* were small genetic lesions, between 1.5 and 6 kb in size, likely to be missed because of the lower resolution of DNA mapping arrays. Similarly, on average, a substantial lower number of CN*Vs* were detected with the DNA mapping arrays compared to those derived from whole exome sequencing data (2.4 *vs.* 9.5, p=0.0345). The power and resolution of our novel statistical tool was illustrated by the detection of acquired MLL-partial tandem duplication (MLL-PTD) lesions in 7 AML patients, which resurfaced in 4 matched relapse cases, and were not detected in the matched control samples. All MLL-PTD lesions were confirmed by standard PCR methodology.
Summary and Conclusions: Our algorithm enables reliable detection of small genetic lesions from different types of NGS data. The analyses based on NGS data revealed that AML has, on average, more genetic lesions than previously thought based on DNA mapping array data. The discordance can be mainly attributed to the increased resolution of NGS data with respect to DNA mapping arrays and the robust removal of systematic noise by our algorithm. Analysis of CN*Vs* derived from whole exome sequencing data reveals a substantial number of cases with MLL-PTD within diagnostic-relapse AML pairs, previously missed with DNA mapping arrays.

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HALOPLEX TARGET DNA ENRICHMENT ALLOWS INVESTIGATION OF COPY NUMBER AND MUTATIONAL STATUS OF KEY GENES IN ACUTE MYELOID LEUKAEMIA WITH NORMAL KARYOTYPE

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Background: Acute myeloid leukaemia (AML) is a deadly haematological malignancy for which individualized treatment based on prognostic stratification is essential to maximize chances of survival. Karyotypic abnormalities carry prognostic significance, but are absent in about 50% of patients (NK-AML), and in this subgroup analysis of gene mutations can be used to stratify patients. Recent advances in AML genomics have defined the set of genes which are recurrently mutated in NK-AML, whilst the prognostic impact of many of these has been determined. Therefore, clinical-grade sequencing platforms will be increasingly useful in clinical practice in the next few years, and this highlights the need for reliable methods targeted gene re-sequencing.
Aims: HaloPlex is a novel, rapid approach for targeted DNA enrichment that requires low amounts of input DNA. We evaluated its performance in detecting abnormalities in coding sequence and copy number of genes recurrently mutated in NK-AML, with special focus on reproducibility and on the quantitative value of data.
Methods: Genomic DNA from 43 NK-AML samples from 40 patients was subjected to Haloplex target enrichment for 24 genes. Target-enriched DNA was sequenced on HiSeq2000, and reads were aligned using BWA. Substitutions and indels were called using standard algorithms and mutations called as previously described (Papaemmanuil *et al.*, Blood 2013).
Results: We sequenced 32.26 gigabases (Gb). A mean of 66.13% mapped on-target (62.94%>74.15%). The mean coverage of the target region was 3674.69x and 91.16% of bases were covered at >30X. Read depth showed significant variability, and coverage across consecutive bases could vary by several folds. This variability was expected as HaloPlex target-enrichment employs digestion of genomic DNA and specific capture of variable-length fragments with subsequent PCR amplification. Nevertheless, we found that this variability in coverage was predictable and highly consistent across samples. Consequently, using normalized data we were able to identify an interstitial deletion of BCOR and three MLL partial tandem duplications. We also report an amplification of KRAS, an event with driver potential in solid cancers but not previously described in AML. The quantitative nature if the data was retained when looking at point mutations, as demonstrated by a narrow range of allelic frequency of heterozygous SNPs. We identified likely oncogenic mutations in 38/40 samples with a median of 3 (1-5) per sample. NPM1 mutations were the most frequent (69%), followed by FLT3 (58%), DNMT3A (35%) and TET2 (32%). As described, NPM1 mutations co-occurred with FLT3 and DNMT3A. Both mono- and bi-allelic FLT3-ITDs were reliably identified. The recurrence rates were consistent with previously published data. We estimated allelic frequency of each mutation and reconstructed the phylogeny of mutations in each. In 2 cases with serial samples we studied the dynamics of tumour evolution and described variants lost and gained at relapse, implying that subclonal evolution can be inferred using Haloplex targeted data.
Summary and Conclusions: Haloplex target enrichment followed by massively parallel sequencing is a simple, rapid and robust method for high throughput screening of gene alterations in NK-AML. It may help prognostic stratification, treatment decisions and minimal residual disease assessment in clinical practice. It can reliably call copy number alterations, substitutions and indels. However, the targeting design should be checked carefully to ensure that the inherent variability in coverage will not affect the efficiency of variant calling.