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ACUTE LEUKEMIAS XVII
Biology and Treatment Strategies

Munich, Germany, February 24– 27, 2019

Free Contributions
Poster Session

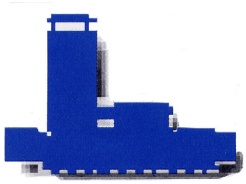
3 Best of Free Contributions

Main Sessions

Meet-the-Professor Session

Satellite Symposia

Late Submissions



International Symposium

ACUTE LEUKEMIAS XVII

Biology and Treatment Strategies

organized by

German AML Cooperative Group (AMLCG)

SFB 1243: Genetic and Epigenetic Evolution of Hematopoietic Neoplasms

and

German Consortium for Translational Cancer Research (DKTK)

together with

Ludwig Maximilians-University Munich (LMU)

German Cancer Research Center (DKFZ)

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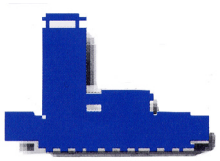

ACUTE LEUKEMIAS XVII
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Contributions of the International Symposium

ACUTE LEUKEMIAS XVII
Biology and Treatment Strategies

February 24 – 27, 2019, Munich, Germany

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Free Contributions
Poster Session

I. AML – BIOLOGY

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(8)Acute Myeloid Leukemia-Associated Mesenchymal Stromal Cells (AMSCS) Support the Growth of AML Cells and Can Be Targeted by Dexamethasone as a Novel Approach to Treat AML

Yahya S. Al-Matary[1,2], Maren Fiori[1,2], Lacramioara Botezatu[2], Dennis Heinrichs[1,2], Aniththa Thivakaran[1,2], Judith Schütte[1,2], Fatema Zamil[2], Daria Frank[1], Pradeep Kumar Patnana [1,2], Ulrich Dührsen[2], Bertram Opalka[2], Georg Lenz[1], and Cyrus Khandanpour[1,2]; [1]Department of Medicine A, Hematology, Oncology and Pneumology, University Hospital Münster, Münster, Germany;[2]Department of Hematology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

(9)The Role of Growth Factor Independence 1 (GFI1) in Leukemia Evolution, Genome Stability and DNA

D. Frank[1,2], Y. Al-Matary[1,2], A. Thivakaran[1,2], J. Schütte[1,2], U. Dührsen[2], and C. Khandanpour[1,2]
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(10)Single Cell Genotyping and Transcriptome Analysis of AML Patients with Subclonal FLT3 Mutations

Johannes W. Bagnoli[1]*, Lucas E. Wange[1]*, Ilse Valtierra[1], Daniel Richter[1], Aleksandar Janjic[1], Johanna Geuder[1], Christoph Ziegenhain[1,2+] and Wolfgang Enard[1+]* ([+] contributed equally)
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D. Hanekamp[1], W. Zeijlemaker[1], A. Kelder[1], G.J. Ossenkoppele[1], G.J. Schuurhuis[1], and J. Cloos[1,2]
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M. Carlet[1], J. Vergalli[1], BC Heckl[1], M. Schmidt-Supprian[2]and I. Jeremias[1,3,4,5,6]
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Gerhard Behre, Franziska Wilke, Johannes Küpper, Alena Renker, Paula Reichelt, and Uwe Platzbecker
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A. González-Sánchez and K. Mills

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D. Richter[1], S. Krauß[1], K.H. Metzeler[2], and W. Enard[1]

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C. Andrés1,2,#*, J. Suárez1,2,#*, N. Dorado4, D. Carbonell2,4, G. Rodríguez4, M. Chicano4, P. Muñiz2,4, M. Bastos4, M. Kwon4, P. Balsalobre4, JL. Díez2,4,10, C. Martínez2,4,#*, and I. Buño1,2,4#*. (*) Identical contribution
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Tània Martiáñez Canales, PhD[1], David C de Leeuw, PhD[1], Eline Vermue[1], Arjo Rutten[1], Fedor Denkers[1], Han JMP Verhagen[1], Jeroen JWM. Janssen, PhD[1], Gert J Ossenkoppele, PhD[1], C. Jimenez, PhD[2], and Linda Smit, PhD[1]
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(32)Simulating Clonal Evolution in Acute Myeloid Leukemia (AML): Bridging the Gap by Mathematical Modelling

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(36)IDH1 Mutation Augments System-Level Metabolic Flexibility that Favors Mitochondrial Oxidative Phosphorylation and Drug Resistance in Acute Myeloid Leukemia

L. Stuani, M. Sabatier, P. Millard, C. Récher, JC. Portais, and JE Sarry
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II. AML – THERAPY

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who Achieve Remission with CPX-351 versus 7+3 Induction

S. Faderl,[1] G.L. Uy,[2] G.J. Schiller,[3] J.E. Cortes,[4] E.K. Ritchie,[5] R.J. Ryan,[1] M. Chiarella,[1] and A.C. Louie[1]

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(40a)Safety and Clinical Activity of Mutant IDH1 Inhibitor IVOSIDENIB (IVO; AG-120) In Combination with AZACITIDINE (AZA) for Newly Diagnosed Acute Myeloid Leukemia (ND AML)

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(41)Molecular Profiling and Outcome of Patients with IDH1/2 Mutated Hematologic Malignancies after Treatment with IVOSIDENIB or ENASIDENIB

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(42)Outcome of Acute Myeloid Leukemia with Inv(3)(Q21q26.2)/ T(3;3)(Q21;Q26.2). Experience of the Spanish PETHEMA and CETLAM Groups

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(43)RAS Mutations Confer an Increased Risk for Relapse in Patients With AML Receiving Induction Chemotherapy

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(44)Do Frailty Scores Help Defining Fitness of Elderly AML Patients at Diagnosis? A Single Centre Study

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(45)Response to Front and Second Line Treatment in Patients with Acute Non Lymphoblastic Leukemia: A Single Center Experience

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(46)Prognosing Efficacy of FLAG Regimen in theTreatment of Relapsed and Refractory AML

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(47)Decitabine as Salvage Therapy after Azacitidine in AML

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(48)MRD Level after Induction Therapy in *NPM1* Mutation and *RUNX1-RUNX1T1* Positive AML Identifies High Risk of Relapse Patients

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(49)Highly Sensitive Residual Disease Detection in Acute Myeloid Leukemia Using Advanced Error Corrected DNA Sequencing

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(50)Sparing Anti-Bacterial Prophylaxis in Acute Myeloid Leukemia during Post Induction Aplasia: Results of a Retrospective Single Center Study

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(51)A Mathematical Model for Relapse Prediction in AML Patients Based on Continuing NPM1 Measurements

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(52)A Mathematical Approach to Assess Duration of Neutropenia within Different Induction Regimens in Acute Myeloid Leukemia (AML)

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(53)Combined Inhibition of the MENIN-MLL Chromatin Complex and FLT3 Acts Synergistically against FLT3 Mutant Leukemias

M.Dzama, M.Taubert, K.Kunz, J.Rausch, CW.Chen, A.Mupo, M.Theobald, T.Kindler, R.P.Koche, GS Vassiliou, SA Armstrong, and MWM Kühn[1]

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(54)Targeting CBP/β-Catenin in MDS/AML

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(55)Extracellular ATP and CD39 Regulates Mitochondrial Function and Cytarabine Resistance through Intrinsic PKA-PGC1α Pathway in Acute Myeloid Leukemia

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(56)Adrenomedullin Receptor CALCRL Drives Drug Resistance of Leukemic Stem Cells in Acute Myeloid Leukemia Extracellular ATP and CD39 Regulates Mitochondrial Function and Cytarabine Resistance through Intrinsic PKA-PGC1α Pathway in Acute Myeloid Leukemia

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(57)Impact of Exchange Transfusion for Prevention of Early Death in a Pediatric APL Patient with Hyperleukocytosis

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III. ALL – BIOLOGY

(58)Prognostic Importance of *IKZF1* Gene Deletions in Patients with Acute Lymphoblastic Leukemia

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(59)Drawing in AF15Q14/CASC5 and APOBEC3A/C in Leukemogenesis

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(60)Inducible Re-Expression of KLF4 Impairs Growth of Patient Derived Acute Lymphoma Leukemia Cells *IN VIVO* and Sensitizes them towards Chemotherapy

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(61)Combined Inhibition of MEK-Signaling and BCL-2 Promotes Synergistic Effects in NRAS-Mutated BCP-ALL Cells

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(62)Heterogenous Activity and Syngergistic Interaction of BH3-MIMETICS

VENETOCLAX, S63845 & A1331852 in B-Cell Precursor Acute Lymphoblastic Leukemia

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(63)RNAi-Screen Identifies Mediators of Stroma-Induced Resistance to NOTCH and mTOR-Inhibition in T-CELL Acute Lymphoblastic Leukemia

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IV. ALL – THERAPY

(64)Trends in Survival of Young Adult Patients with Acute Lymphoblastic Leukemia (ALL) in Sweden and USA

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(65)Young Adults with Acute Lymphoblastic Leukemia: Therapy Optimization in the Republic of Belarus

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(66)Non-Intensive but Non-Interruptive Treatment without High-Dose Blocks is an Effective Strategy for Adult PH-Negative Acute Lymphoblastic Leukemia: The First Interim Results of the Russian Prospective Multicenter RALL-2016 Study

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(67)Monitoring Measurable Residual/Relapsing Disease after Allogeneic Hematopoietic Stem Cell Transplantation in Adult Patients with Acute Lymphoblastic Leukemia

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(68)Next Generation Sequencing as the Way for IGH Earragementmrd Monitoring in B-ALL

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V. IMMUNOTHERAPY

(69)Efficient Elimination of Cells from Patients with Different AML Subtypes by Dual-Targeting Triplebody 33-16-123

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(70)Preliminary Biomarker and Pharmacokinetic-Response Relationships in a Phase 1 Study of AMG 330, a Bispecific CD33

T-Cell Engager (BiTE®) Antibody Construct, in Patients (PTS) with Relapsed/Refractory (R/R) Acute Myeloid Leukemia (AML)

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(71)Enhanced Phagocytic Eradication of Leukemia Cells by Combination of an FC-Engineered CD19 Antibody with CD47 Immune Checkpoint Blockade

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(72)Anti-Human CD117 CAR T-Cells Efficiently Eliminate Hematopoietic Stem and CD117-Positive AML Cells

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(73)ADGRE2 Shows a Favorable Expression Profile for CAR-Targeting in Acute Myeloid Leukemia

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VI. PEDIATRIC ACUTE LEUKEMIAS

(74)NG2 Predictive Value for Presence of *KMT2A*-Rearrangements Differs and Depends on Age and Acute Leukemia Type

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(75)The *MLL*-Recombinome of Pediatric Acute Leukemia in the Russian Federation

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(76)INTRON Retention in KMT2A-MLLT3 Fusion Transcript in Pediatric Acute Leukemia

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(77)Verification of Pediatric *BCR-ABL1*-LIKE ALL Cases by Real-Time PCR

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(78)Efficacy of Epigenetic Therapy with Intensive Chemotherapy in the Treatment of Childhood Acute Myeloid Leukemias

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(79)Analyzing Transcriptional Profiles of Childhood ALL at Single Cell Resolution

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(80)The Definition of the MRD is the Main Stratification Tool for Risk Groups Detection in Children with B-ALL

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(81)Epigenetic Therapy Allows to Improve the Survival of Children with Acute Myeloid Leukemia

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(82)MIR-497~195 Cluster has Tumor Suppressive Function in Pediatric ALL

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(83)*TP53* Alterations and Gene-Expression Profiling of P53 Pathway Genes in Pediatric Acute Myeloid Leukemia

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(84)Hyperleukocytosis in Children with Acute Myeloid Leukemia: A Single-Center Experience

I. Kalinina, O. Goronkova, D. Evseev, T. Salimova, J. Olshanskaya, E. Zerkalenkova, M. Maschan, G. Novichkova, and A. Maschan.
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(85)CD1a and T-CELL Receptors Expression Predicts Outcome in Childhood T-Lineage ALL

A. Popov[1], O. Illarionova[1], A. Chervova[1], T. Verzhbitskya[2], L. Fechina[2], J. Roumiantseva[1], G. Henze[3], and A. Karachunsky[1]
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3 Best of Free Contributions

(Oral presentation in MS IX)

(Poster #3)

The Interruption of PPARγ/RXR FUNCTION BY PML-RARα or TRIB3 Separately Causes Dyslipidemia in Newly Diagnosed APL or in Anti-APL Treated Patients

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(Poster #55)

Extracellular ATP and CD39 Regulates Mitochondrial Function and Cytarabine Resistance through Intrinsic PKA-PGC1α Pathway in Acute Myeloid Leukemia

N. Aroua, ML. Nicolau-Travers, T. Kaoma, N. Bonnefoy, F. Vergez, C. Récher, and JE Sarry

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(Poster #72)
Anti-Human CD117 CAR T-Cells Efficiently Eliminate Hematopoietic Stem and CD117-Positive AML Cells
R. Myburgh[1], J.Kiefer[2], N.F. Russkamp[1], A. Simonis[1], S. Pfister[1], C. Magnani[1], C.M. Wilk[1], A.M. Müller[1], M. van den Broek[3], B. Becher[3], D. Neri[2] and M.G. Manz[1]
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MAIN SESSIONS

Main Session VII

The AML-CG Trials in APL
Eva Lengfelder[1] for the German AML Cooperative Group
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MEET-THE-PROFESSOR SESSION

MEET-THE-PROFESSOR SESSION I
(Gilead Sciences GmbH)

Drug interactions: Putting Success of new Drugs at Risk
Prof. Dr. rer.nat. H.-P. Lipp (PharmD. PhD)
Universitätslinik Tübingen, Tübingen, Germany

SATELLITE SYMPOSIA

SATELLITE SYMPOSIUM I
(Amgen AG)

CD33 BiTE: From Molecule to Therapy
Marion Subklewe
LMU, Klinikum Großhadern, Medizinische Klinik und Poliklinik III, München, Germany

SATELLITE SYMPOSIUM V
(Daiichi Sankyo Oncology Europe GmbH)

Targeted Therapy in AML: What do the Guidelines tell us?
Mark James Levis
Johns Hopkins, Cancer Research Building, Baltimore, USA

SATELLITE SYMPOSIUM VII
(Celgene GmbH)

How Do Genetics of AML Patients Inform Prognosis, Treatment Strategies and Follow-Up Today and Tomorrow?
Konstanze Döhner, Peter Paschka and Hartmut Döhner
Department of Internal Medicine III, University Hospital of Ulm, Germany

SATELLITE SYMPOSIUM VIII
(Pfizer GmbH)

Targeting CD33 in AML
Marion Subklewe
LMU, Klinikum Großhadern, Medizinische Klinik und Poliklinik III, München, Germany

Emerging Treatment Strategies in AML
Carsten Müller-Tidow
Department of Medicine V, Hematology, Oncology and Rheumatology, University of Heidelberg, Heidelberg, Germany

LATE SUBMISSIONS

Progress in AML: The Future
Thomas Schröder
Universitätsklinikum Düsseldorf Abteilung für Onkologie und Klinische Immunologie, Düsseldorf, Germany

FREE CONTRIBUTIONS
Late Breaking Abstract

Outcomes Following Hematopoietic Stem Cell Transplantation in Patients Treated with Chemotherapy with or without Gemtuzumab Ozogamicin for Acute Myeloid Leukemia
E Raffoux[1], J Lambert[2], S Chantepie[3], L Gastaud[4], O Legrand[5], J-P Marolleau[6], C Pautas[7], R Peffault de Latour[8], X Thomas[9], P Turlure[10], RJ Benner[11], E Vandendries[12], K Gogat[13], H Dombret[2], and S Castaigne[2]
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SATELLITE SYMPOSIA

EVENING SYMPOSIUM
(Vector Therapeutics, Inc.)

Immunotherapy for Relapse Prevention in Acute Myeloid Leukemia
Anna Martner, Fredrik Bergh Thorén and Kristoffer Hellstrand
Sahlgrenska Cancer Center, Gothenburg, Sweden

SATELLITE SYMPOSIUM II
(AbbVie Deutschland GmbH)

Welcome and Introduction
Uwe Platzbecker
Universitätsklinikum Leipzig, Hämatologie und Zelltherapie, Leipzig, Germany

Emerging Agents for the Treatment of Patients with AML
Uwe Platzbecker
Universitätsklinikum Leipzig, Hämatologie und Zelltherapie, Leipzig, Germany

From Bench to Bedside: Practical Considerations for the Older Patient with AML
Uwe Platzbecker
Universitätsklinikum Leipzig, Hämatologie und Zelltherapie, Leipzig, Germany

Targeting Apoptosis in AML
Marina Konopleva
MD Anderson Cancer Center, The University of Texas Health Science Center at Houston and The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Strategies to Overcome Resistance to Emerging Agents
Marina Konopleva
MD Anderson Cancer Center, The University of Texas Health Science Center at Houston and The University of Texas MD Anderson Cancer Center, Houston, TX, USA

SATELLITE SYMPOSIUM V
(Roche Pharma GmbH)

MDM2 Inhibition as a New Treatment Approach in AML
Marina Konopleva
MD Anderson Cancer Center, The University of Texas Health Science Center at Houston and The University of Texas MD Anderson Cancer Center, Houston, TX, USA

ACUTE LEUKEMIAS XVII

Biology and Treatment Strategies
Munich, Germany, February 24-27, 2019

FREE CONTRIBUTIONS

ABSTRACTS - Poster Session

(Submitted Abstracts accepted for Presentation in Poster Session; Topics and Numbers correspond to Topics and Numbers in Poster Exhibition)

I. AML – BIOLOGY

1

Clinicopathological Comparison of EGFR Expressing and Non Expressing Acute Myeloid Leukaemia

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Purpose: EGFR, an important proto-oncogene, regulates cell differentiation, proliferation, cell migration and survival in most of the cancer types. The expression and role of EGFR in AML is contradictory which prompted us to compare the clinicopathological markers of AML and carry out a comparative survival analysis between EGFR expressing and non-expressing AML patients (Acute Promyelocytic Leukaemia patients excluded).
Methods: Bone marrow or peripheral blood samples were collected with written informed consent from 60 patients with de novo AML. Real Time Taqman gene expression assays were used for the detection. Statistical analysis was conducted by using SPSS software (IBM SPSS 20).
Results: In our study EGFR expression was detected in 21 out of 60 (35%) patients. FLT3-ITD and AML1-ETO were detected in 3/21(14.5%) and 5/21(24%) patients respectively. Our results showed that there was no significant difference between the EGFR expression and the survival status consisting the follow up time (P = 0.787). FLT3-ITD (P = 0.024), CD14 (P = 0.007) and CD36 (P = 0.061) were associated with poorer prognosis.
Conclusion: Our study has confirmed that EGFR is expressed in human AML. EGFR expression is unlikely to influence the clinical course of AML and nullify EGFR to be a potential drug target in AML.
Conflict of interest: None

2

Unexpected Properties of a Novel T(11;19) out-of-Frame MLL Fusion Gene in Expanding Human CD34+ Blood Progenitor Cells Resembling Acute Myelomonoblastic Leukemia AML M4/5

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Introduction: Acute leukemia with *MLL*-rearrangement (*MLL*-r) is a distinct leukemia subgroup. Despite their dismal clinical outcome, our knowledge about the underlying disease mechanism is still rudimentary. There is increasing knowledge regarding *in-frame* fusion genes resulting in MLL-X fusion proteins. However, the mechanisms and biology of *out-of-frame* MLL fusions, which represent a large group among *MLL* rearrangements, are hardly understood.
Method: Here we have cloned a t(11;19) *MLL* exon 9 and *ENL* exon 2 *out-of-frame* fusion gene containing a 244 base pair intron retention surrounding the chromosomal breakpoint including splice donor/acceptor and branch sites.
Results: Forced expression of this MLL fusion induced rapid outgrowth of retrovirally transduced CD34+ progenitors in IL3, IL6, SCF, FLT3-L, TPO and GM-CSF containing *ex vivo* cultures. This positive selection however did not occur in similarly transduced peripheral blood mononuclear cells. The *ex vivo* CD34+ progenitor culture consistently resulted in myelomonoblastic cells with the ability to form colonies in methylcellulose. The cells arrested at the myelomono-blastic stage and expressed high levels of CD14. Additionally, cytochemical stainings of the non-specific esterase (EST) and peroxidase (POX) resulted in a strongly positive signal for EST compared to a weak reaction for POX, a signature characteristic for AML FAB subtypes M4/M5. To prove the mechanism of splicing we generated an *out-of-frame MLL* fusion gene lacking the splice acceptor site of *ENL*. Cells transduced with this construct did not feature a growth advantage compared to untransduced cells suggesting the correct splicing as an essential mechanism to generate an *in-frame MLL/ENL* fusion gene driving CD34+ cell transformation.
Conclusion: We verified splicing to an *in-frame* fusion transcript via including a GFP tag to the C-terminus of *ENL*. Western blot analyses of MLL/ENL *out-of-frame* transfected cells revealed the presence of a fusion protein and an additional, truncated MLL protein resulting from alternative

splicing. This truncated MLL alone did not trigger CD34+ cell expansion, but potentially promoted outgrowth by cooperating with the MLL/ENL *in-frame* fusion. With this humanized CD34+ model system, we aim to uncover the precise molecular mechanisms of *MLL* rearranged *out-of-frame* fusion genes, the biology of MLL-r acute monoblastic leukemia and ways to interfere with this leukemic entity.
Conflict of interest: None

3

The Interruption of PPARγ/RXR FUNCTION BY PML-RARα or TRIB3 Separately Causes Dyslipidemia in Newly Diagnosed APL or in Anti-APL Treated Patients

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Introduction: Despite dyslipidemia is common in the patients with acute promyelocytic leukemia (APL) in response to anti-APL therapy, the underlying molecular mechanism is to be addressed. Also, it is still unclear for the lipid status in the newly diagnosed APL.
Method and results: Here we conducted a retrospective study to investigate the lipid profile and other major clinical parameters in 120 newly-diagnosed AML patients (60 APL patients versus 60 non-APL AML patients). Overweight (Body Mass Index, BMI > 25) was more in APL patients (52%) than in non-APL AML patients (32%) ($p = 0.02$). Hyperlipidemia was found in 65% (39/60) of APL patients and 36% (22/60) of non-APL patients ($p = 0.0019$). The initial levels of triglyceride (TG) before treatment was high in APL than non-APL patients. Moreover, the total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) in APL patients were all higher than those of non-APL, indicating that a higher proportion of patients diagnosed with APL patients have dyslipidemia in comparison to patients with non-APL AML. Using transgenic mice, we found that APL mice induced by the overexpression of *PML-RARα* had a lipid-metabolic disorder similar to APL patients in comparison with the age-matched wildtype mice. Moreover, there was a negative correlation between the expressions of PML-RARα and PPARγ in human and mice APL cells. PML-RARα inhibited PPARγ activity by interfering with the PPARγ and RXR interaction and promoting PPARγ protein degradation. Interestingly, despite the Arsenic/ATRA therapy degraded PML-RARα protein and restored the expression of PPARγ, this therapeutic formulation induced dyslipidemia in APL patients and mice. We found that the elevated expression of TRIB3, a stress sensor, in response to the arsenic/ATRA therapy suppressed the PPARγ activity by forming a complex of TRIB3/PPARγ/PML-RARα and disrupting the PPARγ/RXR dimer, which resulted in the abnormal lipid metabolism in APL patients. Indeed, PPAR activator enhanced the anti-APL effects but reduced side effects of ATRA/arsenic by improving the dyslipidemia in APL patients.
Conclusion: Our work reveals a critical regulatory role of the PML-RARα/PPARγ/TRIB3 in the dyslipidemia of APL, which suggests a therapeutic rational for combining ATRA/arsenic with PPAR activator for APL therapy.
Conflict of interest: None

4

Role of Transport Proteins and Enzymes in Resistance to Induction Chemotherapy in AML

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Background: A high proportion of patients with AML are refractory to conventional chemotherapy. Even when remission is achieved through chemotherapy, relapse occurs in 60-70% of cases within 5 years. The aim of this study was to investigate whether changes in the expression of genes involved in the uptake/efflux and metabolism of cytarabine and idarubicin (Marin et al. Blood Rev.2016; 30:55-64) are associated with the sensitivity to these drugs of AML cell lines and blast cells obtained from bone marrow of patients with newly diagnosed AML.
Methods: Cytostatic activity was determined in cell lines by the MTT test after incubation for 72 h with cytarabine or idarubicin. Gene expression levels in blast cells were determined by RT-qPCR and associated with the response to induction chemotherapy. Gene expression in cell lines was determined in basal conditions and after 72 h exposure to cytarabine or idarubicin. The functional activity of transport proteins was

determined by flow cytometry using specific substrates and inhibitors.

Results: The order of chemoresistance to cytarabine in the cell lines was (from less to more sensitive) K-562>>MOLM-13≈HEL>HL-60 and to idarubicin K-562>HEL>MOLM-13≈HL-60. The highest levels of *ENT*s and *CNTs* were found in MOLM-13 cells and the lowest in K-562. Expression of *MDR1* was high in HEL cells and undetectable in HL-60 cells, while expression of BCRP was HEL>K-562>MOLM-13>HL-60. Higher levels of the enzyme that activates cytarabine, *dCK*, were found in HEL and MOLM-13, however, levels of *CDA*, an enzyme that inactivates cytarabine were higher in MOLM-13 than in the rest of the cell lines. Over-expression of several ABC proteins was found after exposure of cell lines to idarubicin, but not to cytarabine. Flow cytometry studies demonstrated that MRP1-4, but not MRP5, were functional. A significantly higher expression of *MDR1* and the inactivating enzymes *CDA* and *5-NT* was found in AML patients with primary refractory disease, but not in patients with complete remission after one or two cycles of chemotherapy.

Conclusion: The expression of plasma membrane transporters involved in cytarabine/idarubicin uptake/efflux and enzymes may affect the response of leukemia cells to these drugs. This analysis might provide complementary information to clinical outcome of AML patients according to their risk stratification by genetics.

Conflict of interest: None

5

Targeting the Methyltransferase Activity of NSD1 in NUP98-NSD1 Positive Leukemia as a Novel Therapeutic Strategy

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Purpose: NUP98-NSD1 positive acute myeloid leukemia (AML), defined by NUP98-NSD1 translocation t(5;11)(q35;p15.5), is a subgroup within pediatric and adult AML with poor prognosis (Thol et al., 2013). As a result of the NUP98-NSD1 fusion, NSD1 is constitutively expressed and causes H3K36 methylation of HOXA genes, which contributes to myeloid progenitor cell immortalization and leads to AML. We hypothesized that inhibition of methyltransferase activity of NSD1 could be an effective treatment strategy for NUP98-NSD1 AML patients. We assessed the efficacy of NSD1 inhibitor suramin in a preclinical patient-derived xenograft (PDX) model of NUP98-NSD1 leukemia.

Methods: In order to screen NUP98-NSD1 positive AML patients, nested PCR was performed. We identified a patient with NUP98-NSD1 fusion in whom the 11th exon of NUP98 is fused to the 6th exon of the NSD1 gene. Bone marrow cells from this patient were serially transplanted into NSG (NOD-scid IL2Rgamma^{null}) mice. Leukemia development was monitored by engraftment of human CD45+ cells in peripheral blood and blood counts. Fifteen days after transplantation, 9 mice were treated with 10 mg/kg suramin (2 days/week for 10 weeks) and 9 mice were treated with control solvent.

Results: Ten weeks after transplantation of the patient cells, the engraftment of human leukemia cells reached 90% and mice succumbed to leukemia after 90-120 days. In successive transplantations up to the 6th generation, the NUP98-NSD1 fusion was confirmed in leukemic cells, supporting the importance of the fusion for leukemia development and stability of the model. While mean human leukemic cell engraftment was similar between the control and treatment groups at the start of suramin treatment (0,51% (N=9) and 0,71% (N=9), respectively), the mean leukemic cell engraftment was significantly lower in treated mice after one and two months of treatment (one month: CTRL, 4,8%; treated, 2,66%, P= 0.10; two months: CTRL, 87,3%; treated: 66,5%, P= 0.016). Additionally, suramin prolonged the median survival of mice significantly compared to control mice (126 vs 114 days after transplantation, P=0.008).

Conclusion: We show that blocking the NSD1 methyltransferase activity can delay leukemia development in vivo and prolong the survival of mice carrying a NUP98-NSD1 positive AML. Suramin is an approved drug used in the treatment of African trypanosomiasis and has been evaluated in clinical trials of other cancers like prostate cancer and bladder cancer. Combination studies with suramin are ongoing in our lab to develop improved treatment options for patients with NUP98-NSD1 fusions.

Conflict of interest: None

6

Disruption of CSF-1R Signaling Inhibits Growth of INV(16) AML

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Introduction: Inversion of chromosome 16 as single cytogenetic abnormality occurs in 5-7% of de-novo Acute Myeloid Leukemias (AML) and correlates with a comparatively favorable outcome and a high rate of complete remissions (CR) after conventional cytotoxic therapy. Nevertheless,

nearly half of the patients eventually relapse, emphasizing the need for novel targeted treatment options and a more profound understanding of the causal pathophysiology. Until recently, however, approaches to study this cytogenetically defined disease entity were limited by e.g. the minimal engraftment of inv(16) AML samples in mouse xenotransplantation models.

Method: We previously defined the absence of M-CSF signaling as limiting factor for engraftment of inv(16) AML in immunodeficient mice and established human cytokine knock-in mice with expression of human M-CSF (MITRG-SKI) as superior xenograft model (Ellegast et al., Blood 2016). We here present data that further substantiate the dependency of inv(16) AML on M-CSF-signaling and indicate that targeting of the cognate receptor CSF-R1 by small molecule inhibitors could be therapeutically utilized.

Results: First, protein expression of CSF-R1 was demonstrated in an inv(16)(p13q22) AML cell line (ME-1) as well as in primary patient samples. Supplementation of M-CSF in long term in-vitro culture provided a survival benefit of bulk inv(16) AML cells which could be abrogated by a monoclonal antibody against M-CSF (MCS110). In contrast, no comparable effects were seen on AML cells without inv(16). In vitro treatment with a small molecule inhibitor of CSF-R1 reduced the viability of inv(16) AML cells even more drastically. Mechanistically, CSF-R1 inhibition resulted in reduced phosphorylation of CSF-R1 as well as the downstream kinases MAPK and Akt in primary AML cells. Finally, in vivo efficacy of the CSF-R1 inhibitor as single agent was demonstrated using the established xenograft model of inv(16) AML in MITRG-SKI mice. Following 21 days of treatment in a therapeutic setting, human engraftment was reduced by 40% with no apparent hematopoietic or non-hematopoietic toxicity.

Conclusion: In conclusion, these data further corroborate the dependency of inv(16) AML on the growth factor M-CSF and its cognate receptor CSF-R1. Intriguingly, CSF-R1 inhibitors have demonstrated a favorable safety profile, making them promising candidates for targeted treatment of inv(16) AML in combination with conventional agents in future clinical trials.

Conflict of interest: None

7

Identification of Genetic, Epigenetic and Cellular Events Implicated in Distinct Clinical Outcomes of DNMT3A^{mut} AMLS

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Introduction: Acute myeloid leukemia (AML) originates from mutations in multipotent hematopoietic stem cells (HSCs). Current therapies target the leukemic bulk cells while sparing leukemic stem cells (LSCs), which originate relapse. AML patients frequently carry mutations in the *DNMT3A* gene, one of the initiating lesions from which AML evolves. However, the identity and order of the subsequent mutations are poorly understood. Moreover, patients with *DNMT3A^{mut}* can have diverse clinical outcomes with some suffering from early relapse (ER) while others undergo long-term remission (LTR). To address this phenomenon, we perform functional and multi-omics assays to identify the causes leading to either ER or LTR of DNMT3A^{mut} AML.

Method: We transplanted 39 diagnostic AML samples from patients with DNMT3A R882^{mut} into NSG mice. After 12-16 weeks post transplantation the degree of human engraftment in the bone marrow was determined. Moreover, we characterized the diagnostic samples using 13 stem cell associated cell surface markers.

Results: DNMT3a R882^{mut} patient samples showed high engraftment potential with 92% of the samples engrafting. All samples from ER group (n=12) initiated leukemic engraftment, while multilineage engraftment was observed only in LTR AMLs (n=9 out of 27). This suggests a suppression of detectable normal HSC activity in more aggressive AMLs. We confirmed that CD34 expression is decreased in the good prognostic group. Likewise, the CD34+38- cell frequency is increased in the relapse group. The ER was also associated with increased CD123 and 45RA expression. Although the gene expression profiles of patients in both groups are very similar, the ER patients are characterized by the increased expression of *stemness* genes (CD34, WNT, ...).

Conclusion: To better characterize LSCs, we will FACS-sort the primary samples based on the expression of CD34, GPR56 and NKrelated ligands. Cell fractions were be subjected to i) xenotransplantation; ii) Transcriptome analysis, ATAC-sequencing and mutational profiling. This multi-omics approach linked to functional LSC analysis will help to identify differentially expressed genes/proteins and enhancer regions in LSCs in AML patients with distinct outcomes.

Conflict of interest: None

8

Acute Myeloid Leukemia-Associated Mesenchymal Stromal Cells (AMSCS) Support the Growth of AML Cells and Can Be Targeted by Dexamethasone as a Novel Approach to Treat AML

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Introduction: Acute myeloid leukemia (AML) is a malignant disease characterized by poor prognosis. We investigated the interaction between AML cells and surrounding stroma cells in the bone marrow (BM). Mesenchymal stromal cells (MSC) are an important element in BM stroma.
Result: MSC from AML patients or leukemic mice (AML-associated MSCs (AMSCs)) supported the in vitro growth of AML cells better than MSCs from healthy controls. AMSCs not only shift the cell cycle toward the proliferating stages but also protect the leukemia cells against apoptosis. *In vivo*, we observed an increase in numbers of MSCs and other stromal elements in the BM of leukemic mice. RNA microarray of AMSCs showed an enrichment of pathway governing Notch signaling as well as a pro-inflammatory state in the MSC. We examined whether this pro-inflammatory state can be targeted by dexamethasone. Treatment with dexamethasone abrogated the AML supporting function of AMSC *in vitro*, and significantly prolonged survival of leukemic mice *in vivo* with a lower frequency of MSCs and other stromal elements in leukemic mice. As a possible link, we observed that adding dexamethasone leads to downregulation of upregulated Notch signalling in AMSC. **Conclusion:** In summary, MSCs support the growth of AML cells *in vitro* and *in vivo*, and dexamethasone could be a novel approach to treat AML.
Conflict of interest: None

9

The Role of Growth Factor Independence 1 (GFI1) in Leukemia Evolution, Genome Stability and DNA

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Introduction: Growth factor independence 1 (GFI1) is a transcriptional repressor, which regulates the proliferation and differentiation of hematopoietic stem cells. We have previously shown that lower expression of GFI1 promotes the development of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) in patients and murine models. Additionally, a single nucleotide polymorphism variant of GFI1, called GFI1-36N (Ser36 replaced by Asn36) contributes to the development of MDS and AML by failing to induce certain genome-wide epigenetic changes. AML patients with low levels of GFI1 or GFI1-36N can be detected in around 30% and have poor prognoses.
Moreover genomic instability is one of the key players in the development of AML.
Method and results: Our group wants to investigate whether GFI1 regulates the genomic stability of leukemia. Therefore we reanalyzed available genomic data of MDS/AML patients with expression of GFI1-36N. These patients showed significantly more cytogenetic aberrations than patients with normal level of GFI1-36S. Additionally, we observe that leukemic mouse cells with low level of GFI1 or expression of GFI1-36N showed significantly higher rate of genomic alterations such as mutations, deletions and insertions than GFI1 wt expressing murine leukemic cells. These findings indicate that low expression of GFI1 or expression of GFI1-36N is associated with genomic instability. Therefore we investigated the role of GFI1 in DNA-repair. We irradiated GFI1 wt, GFI1 low expressing and GFI1-36N expressing thymocytes. Using comet and γH2AX assay we showed that presence of Gfi1-36N or reduced level of GFI1 is associated with diminished DNA repair. Furthermore we found that presence of GFI136N or reduced level of GFI1 is associated with low level of MGMT. Other groups have shown that patients with low expression of MGMT respond well to treatment with Temozolomide (alkylating agent). Due to this fact we treated leukemic cells differently expressing GFI1 with Temozolomide. We found that the leukemic cells with low level of GFI1 or GFI1-36N are highly susceptible to Temozolomide compared to the leukemic control cells.
Conclusion: In summary, low expression of GFI1 or expression of GFI1-36N leads to a clonal genetic evolution by impeding DNA repair. Therefore GFI1 could be a new target in leukemia therapy by using Temozolomide for these patients.
Conflict of interest: None

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Single Cell Genotyping and Transcriptome Analysis of AML Patients with Subclonal FLT3 Mutations

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Introduction: Single-cell RNA sequencing (scRNA-seq) has emerged as a central genome-wide method to characterize cellular identities and processes. Unfortunately, the underlying genetic causes for transcriptional heterogeneity cannot be resolved by transcriptome analysis alone. However, the combination of genotype and transcriptome measurements holds great promises to understand the impact of clonality in disease progression as well as treatment success.
This is especially true for acute myeloid leukemia (AML) as many patients show a variety of subclonal mutations rather than a shared disease marker mutation. One of these mutations is an internal tandem duplication (ITD) in fms like tyrosine kinase 3 (FLT-3) leading to a constitutively active kinase and worse prognosis for disease progression. In order to better understand the transcriptomic programs of cells harbouring this mutation, we used mcSCRB-seq to generate scRNA-seq data for over 5000 cells from 9 AML patients with subclonal FLT3-ITD mutations.
Aims and method: To address the detection of genetic variants, we developed a new protocol making it possible to combine sensitive and accurate transcriptome measurements with genotyping in the same cell. Using this approach we call scTAG-seq (single cell transcriptome and genotyping), we can group cells by specific mutations, and jointly analyze the impact of the genotype on the corresponding transcriptome. This enables us to discover intra- and inter-patient changes as a result of the FLT3-ITDs. To facilitate the design and analysis of scTAG-seq experiments, we provide a dedicated primer design tool and a preprocessing pipeline.
Conclusion: Conveniently, scTAG-seq libraries can be generated a posteriori from already existing scRNA-seq libraries and the method is generally applicable to most high-throughput scRNA-seq methods.
Conflict of interest: None

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CD34+CD38- Leukemic Stem Cell Load at Diagnosis Predicts Outcome in Acute Myeloid Leukemia

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Introduction: Present risk stratification protocols in acute myeloid leukemia (AML) based on cytogenetic, molecular and clinical parameters still imperfectly predict disease outcome. We and others have previously shown that flow cytometry minimal residual disease detection (MRD), as well as leukemia stem/progenitor cell MRD detection (Zeijlemaker et al, 2018; Bradbury et al, 2015) are independent prognostic indicators, important for accurate adaptation.
Aim and method: As this is only applicable when patients achieve complete remission we have studied the possible use of leukemic stem cell (LSC) frequency at time of diagnosis for prediction of outcome. CD34+CD38- LSC were measured by flow cytometry at time of diagnosis (n=594). Patients were categorized based on previously defined cut-off (Terwijn et al, 2014) of 0.03% to discriminate LSClow and LSChigh cases.
LSClow samples were associated with good molecular aberrancies ($FLT3^{wt}/NPM1^{mut}$) and LSChigh samples with poor ($FLT3^{mut}/NPM1^{wt}$, EVI1 over-expression) molecular aberrancies. To establish the prognostic value of LSC, univariate analysis was performed which showed a significant decrease in overall survival (OS) with increasing LSC frequencies.
Results: Multivariate analysis including molecular aberrations, HOVON risk classification, age and white blood cell count, revealed LSC frequency as independent prognostic factor for OS, with hazard ratio (HR) 1.94 (95%CI 1.11-3.39) in LSClow and 2.38 (95%CI 1.34-4.25) in LSChigh, relative to the CD34-negative group (patients with no malignant CD34+).
Conclusion: At time of diagnosis CD34+CD38- LSC frequencies were correlated with OS. The association with molecular aberrancies suggests that these and possibly other genetic aberrations may translate into differences in frequencies of CD34+CD38- LSC. Therefore, CD34+CD38-LSC frequency may represent the total of effects of all mutations that influence therapy resistance, important for adjustments in current diagnosis risk models.
Conflict of interest: None

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Whole Exome Sequencing Identifies Recurrent Somatic Mutations in Upstream Open Reading Frames (uORFs) In Acute Myeloid Leukemia

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Introduction: Upstream open reading frames (uORFs) are cis-regulatory elements in the 5´-transcript leader regions of eukaryotic mRNAs and often regulate translation of the associated downstream protein coding sequences. Both, the genetic gain or loss of uORF function has been linked to human disease, yet the prevalence and functional impact of uORF-associated mutations in hematologic malignancies is unknown.
Method: Here, we determined the genetic variability at uORF initiation and termination codons in a cohort of 162 patients suffering from acute myeloid leukemia (AML) by analyzing whole exome sequencing data obtained from The Cancer Genome Atlas consortium. Mutect2-generated variant call format files were computationally screened for genetic variation at the 59818 uORF initiation and 47108 corresponding uORF termination codons of the human genome. Minimal requirements for a positive call were ≥10 total reads, ≥3 alternative reads (alt) deviating from the reference base (ref), and the alt/ref ratio being ≥4-times higher in the tumor vs. the normal control sample.
Results: The exome sequencing datasets (predominantly focusing on the protein coding exome) covered on average 35.6% (±5.8% SD) of the targeted uORF codons by ≥10 total reads. With a median sequencing depth of 108 reads recurrent somatic mutations in ≥3 patients were detected at 7 uORF initiation- and 13 uORF termination codons. One of the uORF mutations recurred in eight patients (5% of the cohort), one in seven patients (4.4%), three mutations were observed in five patients (3.1%), two in four patients (2.5%), and 13 mutations were detected in three patients (1.9%). The uORF-associated variants affected several regulatory genes, including DNAJB5 (chromatin remodeling), TRMT9B (tumor suppressor), CAPRIN2 (erythroblast differentiation and Wnt-signaling), RBBP4 (E2F-associated cell cycle regulation) and the growth hormone receptor GHR (JAK2/STAT5-signaling).
Conclusion: These data reveal substantial genetic variability at uORF initiation and termination codons in human AML. Our results warrant further functional analyses of the newly identified uORF-associated variants to characterize the pathogenic impact of defective uORF-mediated translational control in malignant transformation, response to treatment and the overall outcome of AML patients.

Conflict of interest: None

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MCL1 Plays an Essential Role for Patients'AML, as Shown by Inducible Knockdown in PDX Models *IN VIVO*

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Introduction: The anti-apoptotic protein myeloid cell leukemia 1 (MCL1) is frequently overexpressed in cancers including acute myeloid leukemia (AML) and plays an important role in tumor development. Here we asked, whether MCL1 might represent a suitable target for anti-cancer treatment and might play an essential function for patients' AML cells growing in vivo.
Aim and method: We aimed working with a highly clinic-related and molecular in vivo model, in the complex environment of individual tumor cells. We developed a novel technique which enables inducible knockdown in patients' AML tumor cells combined with tightly controlled competitive in vivo assays. We grew primary patients´AML cells on NSG mice to generate patient-derived xenografts (PDX) and used lentiviruses for their genetic engineering. Control and MCL1 shRNAs were cloned in an inducible way and connected to distinct fluorochrome markers. For competitive in vivo assays, mice were injected with a 1:1 mixture of sh-control and sh-MCL1 cells; at different disease stages, mice were systemically treated to induce expression of both shRNAs and color distributions were measured by flow cytometry to estimate the in vivo effects at the end of the different incubation periods.
Results: AML PDX cells with knockdown of MCL1 revealed a marked growth disadvantage compared to cells with control knockdown within the same animal. MCL1 was essential and required for different AML PDX models obtained from different patients with different cytogenetics and risk factors. Induction of MCL1 knockdown severely reduced AML PDX fitness at all disease stages, from minimal disease to advanced disease.
Conclusion: Our data show for the first time that patient derived AML cells growing in vivo rely on

MCL1 and indicate that MCL1 represents an attractive therapeutic target, fostering development of drugs targeting MCL1 for treatment of AML patients. Our novel design of pre-clinical molecular trials identifies essential therapeutic targets for individual patient's AML in vivo, closing an existing gap. Targets with molecularly validated essential function will improve future drug development and clinical trials on personalized therapies.
Conflict of interest: none

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From Cellular Therapies to RNA-Based Approaches against Leukemia: Disturbance of the C/EBPA-MIR-182 Balance Impairs Granulocytic Differentiation and Promotes Development of Acute Myeloid Leukemia

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Background: Transcription factor C/EBPα is a master regulator of myelopoiesis and its inactivation is associated with acute myeloid leukemia. Deregulation of C/EBPα by microRNAs during granulopoiesis or acute myeloid leukemia development has not been studied.
Results: Here we show that oncogenic miR-182 is a strong regulator of C/EBPα. Moreover, we identify a regulatory loop between C/EBPα and miR-182. While C/EBPα blocks miR-182 expression by direct promoter binding during myeloid differentiation, enforced expression of miR-182 reduces C/EBPα protein level and impairs granulopoiesis in vitro and in vivo. In addition, miR-182 expression is highly elevated particularly in acute myeloid leukemia patients with C-terminal CEBPA mutations, thereby depicting a mechanism by which C/EBPα blocks miR-182 expression. Furthermore, we present miR-182 expression as a prognostic marker in cytogenetically high-risk acute myeloid leukemia patients.
Conclusion: Our data demonstrate the importance of a controlled balance between C/EBPα and miR-182 for the maintenance of healthy granulopoiesis. Subsequently, we will discuss strategies how to use microRNAs to develop novel RNA-based approaches against leukemia
Conflict of interest: none

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Gene Fusion Detection by RNA-SEQ In Acute Myeloid Leukemia (AML)

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Introduction: Accurate and complete classification of the AML subtype in a patient is a crucial step for the prediction of outcome and the treatment strategy. Identifying the spectrum of genetic abnormalities by Polymerase chain reaction (PCR), Cytogenetics and Fluorescence In Situ Hybridization (FISH) in routine diagnostics is labor intensive, not systematic and error-prone due to subjective assessment. In contrast, next-generation sequencing approaches are able to automatically and reliably identify mutations, insertions, deletions and copy number alterations at the single nucleotide resolution. Furthermore, recent developments of methods for the detection of gene fusion transcripts employing RNA sequence data seem to provide robust results.
Aim and method: To assess the performance of two gene fusion detection algorithms (FusionCatcher, Arriba), we analyzed the transcriptomes from 396 well-characterized AML samples, of which 49 had a matched remission sample, and 38 publicly available healthy control samples. According to Cytogenetics, 84 out of 396 (21%) samples harbored translocations of which 47 samples were further analyzed by FISH or/and PCR confirming the observed translocations.
Results: Fusion detection by the means of RNA-Seq showed evidence for fusion genes corresponding to the reported translocations in 52 out of 84 (62%) samples. Furthermore, 16 out of 396 (4%) samples were found to harbor known pathogenic fusions, described in previous studies, which were not reported by routine diagnostics, FISH or PCR: *CBFB-MYH11*, *RUNX1-RUNX1T1* and *DEK-NUP214* were found each in two samples; *NUP98-NSD1* was found in eight samples; *RUNX1-CBFA2T2* and *RUNX1-CBFA2T3* were found each in one sample. Pending reanalysis of those samples by routine diagnostics shall confirm the newly detected fusions. Additionally, we detected four novel fusion genes (*BCOR-STAG2*, *NMI-THEMIS*, *FAM69B-POLR3B*, *ZNRF2-TMPRSS9*) with high evidence which remain to be validated by complementary methods.
Conclusion: In our study, we have not only demonstrated that the application of RNA-Seq to the detection of fusion genes is a valuable complement to diagnostic routine but also has the

potential to discover novel putatively pathogenic fusions.
Conflict of interest: none

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GATA2 ZF1 Mutants Affect Erythroid Differentiation and Impact on Chemotherapy Response

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Introduction: The transcription factor GATA2 plays an important role in cell lineage decisions during hematopoiesis. *GATA2* is highly expressed in hematopoietic progenitors and stem cells, whereas its expression declines after erythroid commitment. Downregulation of *GATA2* is therefore required for erythroid differentiation.
Method: We generated stable K562 cell lines using retroviral transduction with MSCV-IRES-GFP (pMIG) constructs encoding GATA2 WT, the GATA2 ZF1 mutant A318T and GATA2 ZF2 mutant L359V.
Results: In a proliferation assay, we observed that K562 cells expressing the GATA2 WT show a growth disadvantage when compared to cells expressing GATA2 ZF1 and ZF2 mutants.
Furthermore, RNA-seq data brought forth differentially expressed genes when comparing cells expressing GATA2 WT vs. mutants, amongst others *DACH1* and *PRG2*. It was previously shown by others that ectopic expression of DACH1 contributed to the maintenance of clonogenic activity and blocked the differentiation of myeloid progenitors. PRG2 function is related to drug resistance and it is an indicator of poor prognosis in AML.
Additionally, we showed that K562 cells overexpressing GATA2 ZF1 mutant A318T are more sensitive to Daunorubicin treatment, whereas GATA2 ZF2 mutant L359V seems to be more resistant when compared to WT. Current patient data suggests that A318T is more likely to be lost during disease progression, whereas ZF2 mutations may promote chemotherapy resistance.
We are currently performing differentiation-assays using hCD34+ expressing GATA2 WT, the GATA2 ZF1 mutants A318T and G302D and GATA2 ZF2 mutant L359V. Preliminary results showed that the GATA2-mediated block of erythroid differentiation is rescued by the ZF1 mutants whereas the ZF2 mutant may aggravate the block.
Conclusion: Although the underlying mechanism remains elusive, the GATA2 ZF mutations tested cause an overall disruption of normal hematopoietic differentiation and may have further implications in chemotherapy resistance. Our results suggest distinct roles for individual GATA2 mutations depending on the altered ZF domain.
Conflict of interest: none

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ZBTB7A Mutations in Acute Leukemia Deregulate Lineage Commitment

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Background: ZBTB7A is a transcription factor involved in the regulation of hematopoietic linage fate decisions. Recently, we found ZBTB7A to be specifically mutated in 23% of Acute Myeloid Leukemia (AML) patients with translocation t(8;21), harboring the RUNX1/RUNX1T1 fusion gene. We hypothesized that loss of ZBTB7A function may be necessary for a complete block of differentiation in AML t(8;21).
Aim and method: To study the role of ZBTB7A in myeloid differentiation, we transduced human CD34+ cells with ZBTB7A wild-type (WT) or its mutants (R402C and A175fs). In colony formation assays, ZBTB7A WT expression resulted in a reduced number of colonies compared to the Empty Vector (EV) transduced control cells (36.5 vs. 80). For the mutants R402C and A175fs colonies numbers similar to EV were obtained (119 and 78.5). In WT expressing cells, no erythroid colonies were observed. These data suggest a loss of the anti-proliferative effect and rescue of erythroid differentiation upon ZBTB7A mutation.
Secondly, we directed hCD34+ into the monocytic/granulocytic linage in a liquid culture. We then assessed differentiation by flow cytometry using the markers CD15 (granulocytic) and CD14 (monocytic).
Results: Cells transduced with EV presented a total of 39.76±2.7% (Mean ±SEM) undifferentiated cells. When transduced with WT, the percentage of undifferentiated cells increased to 73.26±6.34%. Transduction with R402C or A175fs delivered similar trends as EV transduced

cells, 57.33±9.19 and 41±11.69%, respectively. Additionally, we differentiated the cells into erythrocytes and assessed differentiation by means of the early erythroid marker CD71, and late erythroid marker CD235a. EV presented 59.73 ±16.77% terminally differentiated cells, while WT cells only differentiated up to 21.49±10.17%. R402C and A175fs showed 53.5±17.25 and 60.93±21.81%, respectively.

Conclusion: These data support a regulatory role of ZBTB7A in linage commitment and a loss of function phenotype for the mutations found in AML t(8;21). Further studies are needed to understand the cooperation between RUNX1/RUNX1T1 and ZBTB7A mutation during leukemogenesis.

Conflict of interest: none

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Tyrosine Kinase Activity Array Technology Reveals the Effect of DASATINIB on Acute Myeloid Leukaemia Cell Signaling Networks

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Purpose: SRC family kinases are important regulators of cell growth whose activity is altered in many cancers including leukaemia. Previous results from our group have shown that Src inhibitors affect the cell cycle in acute myeloid leukaemia (AML) cell lines but their effect is possibly due not only to Src inhibition but also to inhibition of other related kinases. To expand our investigation of the changes in signalling induced by the Src inhibitor, Dasatinib, in AML cells we have used a new kinase activity array technology. Identifying the differences in kinase response to Dasatinib across AML cell lines could give insight into the potential of targeting Src isoforms for the treatment of specific subsets of patients.

Method: The Tyrosine Kinase PamChip® Arrays, processed in PamStation®12, were used in this study. AML cell lines, representing the spectrum of disease sub-types, were treated with Dasatinib and samples were processed following the manufacturer's protocol. Image analysis and subsequent data analysis were performed using proprietary PamGene® software (BioNavigator63 and Sample Annotator) and with other bioinformatics tools such as Ingenuity Pathway Analysis (Qiagen®) used for biological interpretation.

Results: Dasatinib produced activity changes in around 70% of the 196 tyrosine kinases represented in the arrays by 2 hours post treatment, and that effect lasted for at least 24 hours. A small number of tyrosine kinases, around 2% of the total, showed an increase in activity. Some of the affected kinases are implicated in metabolic, cell cycle regulation and adhesion pathways. As expected, Src family members were inhibited by Dasatinib, but with different effectiveness and trajectories.

Conclusion: Specific Src family members and their pathways are potential targets for treatment in particular subsets of AML patients. The analysis of gene expression data can be useful for identification of such groups of patients; however, they cannot determine kinase activity and the response to inhibitors. In this regard, the Tyrosine Kinase PamChip® Array technology is a powerful tool to investigate tyrosine kinase activity in an accurate, high-throughput manner.

Conflict of interest: none

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Treatment of rMLL^{+} AML by Simultaneous Inhibition of LSD1 and MEK Pathway

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Background: While methylation and acetylation of lysine residues of histone proteins have been extensively studied, little is known about histones phosphorylation in leukemia.

Aim and method: We have previously shown that inhibition of LSD1 exerts a potent antileukemic effect on the poor prognosis MLL-AF9^{+} AML. The activity of the histone demethylase LSD1 has been shown to be prevented by phosphorylation of histone H3 at threonine 6 (H3T6) by protein kinase C beta I (PKCbI) during androgen-dependent kinase signaling activation in prostate cancer. The phosphorylation of LSD1 itself also plays a role in metastatic events in solid tumors and modulate its binding with other cofactors to target promoters during physiological inflammatory response. Hereby we aim to assess the effect of chromatin phosphorylation on response to LSD1 inhibition therapy of AML.

Results*:* LSD1 inhibition (LSD1i) led to induction of a TLR-pathway dependent immune response as well as Nf-KB / MAP kinases signalings (NES=2.14, nom. P<0.001, FDR 0.0 and NES=1.87, P=0.0, FDR= 0.18, respectively) (RNAseq analyses). We therefore assessed the status of the MAPK-pathway upon LSD1i. Intriguingly, we reported increase of phosphorylated ERK in the nucleus of leukemic cells upon 48h of treatment with LSD1i, while level of phosphorylated Akt remained comparable to control cells. We then tested the effect of combining LSD1i with MEK-, mTOR-, and GSK3-inhibitors in leukemic cells. After 5 days cells treated with combination of MEK- and LSD1 inhibitors displayed significant decreased proliferation in comparison to the single drug

treatment (IC50 MEKi= >1μM, LSD1i= ~1μM, MEK1+LSD1i= ~1 nM). In contrast, we reported no synergistic benefit of combination of LSD1 with mTOR- and GSK3-inhibitors. This is in line with the hypothesis previously suggested about the main role of the MAPK pathway in rMLL+ AML. Furthermore, we assessed the effect of inhibition of MAPK-activators such as PKCs, via treatment with 1-octadecyl-2-O-methyl-glycero-3-phosphocholine (ET-18-OCH3) and Bisindolylmaleimide VIII (BIM-8) in combination with LSD1i. After 4 days of exposure to this combination treatment we reported increased proliferation in comparison to the LSD1i-single treated cells. This suggests that balanced activity of PKC is necessary to mediate the antileukemic effect of LSD1, maybe by phosphorylating LSD1 or MAPK-targets. Preliminary Western blot assays on histones extracts upon LSD1i indicate imbalanced phosphorylation of threonine 11 and threonine 6 of histone 3.

Conclusions: Combination therapy with LSD1i and MEKi might represent a promising treatment option for $rMLL^+$ AML. Further molecular investigations will allow a closer look into the histone phosphorylation profile in this AML subset.

Conflict of interest: none

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Irradiation of Microenvironment Neutralizes Epigenetic Therapy by LSD1 Inhibition in Murine AML Model

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Introduction: A large proportion of AML patients relapse after standard therapies and the persistence of leukemic clones might be the result of interaction with the microenvironment. Previously it has been shown that malignant cells induce an acute inflammatory response on mesenchymal stromal cells (MSC), and that MSC are involved in resistance to therapy and in tumor cells / bone marrow (BM) microenvironment interactions. The irradiation, which is included in treatment protocols for solid tumors and is used for conditioning regimens for BM transplantation (BMT), has been shown to induce release of multiple cytokines from endothelial cells and in some case to promote metastasis.

Aim: Hereby we aimed to investigate the effect of microenvironment changes upon irradiation on response of leukemia to epigenetic targeted therapy such as inhibition of the lysine-specific demethylase 1 (LSD1i).

Results: Mice treated with LSD1i showed a significant reduction of engraftment in the BM compared to the untreated mice, as expected (21,6% vs. 80%, respectively, $p<0.0001$). This treatment led to upregulation of immune relevant genes (*Cd86, Irf8, Tlr8, Mmp8, Mmp27, Mmp19*). In parallel, we observed that mice, which underwent irradiation before BMT, did not respond to the treatment (83,8% in untreated vs. 87,6% in treated, $p=0.63$). Immunophenotypical analyses revealed a persistence of more primitive $ckit^+$ cells in the BM of irradiated resistant mice, while the BM-derived mivroenvironment of irradiated recipients displayed an enrichment in $Mac1^+$ and $Sca1^+$ cells. We further assessed the resistance phenotype observed *in vivo* using irradiated and non-irradiated S17 feeder cells *in vitro*. The proportion of living leukemic cells treated with LSD1i in the presence of supernatant (SN) from S17 cells (S17i=irradiated and S17ni=non-irradiated) was 3fold higher than the proportion of living cells treated in the absence of S17-SN (30,64% S17i, 30,11% S17ni vs 10,68% without S17, respectively). Interestingly, also in coculture experiments we reported that the proportion of proliferating cells was higher when they were treated with LSD1i on S17i (29,1% vs. 11,9% on S17ni and 1,6% leukemic cells alone), with persistence of blast colony-forming units after treatment with LSD1i during co-culture with irradiated feeder cells.

Conclusions: We observed that the irradiation of the host leads to neutralization of the anti-leukemic effect of LSD1 inhibition *in vivo*. *In vitro* experiments suggest that a different response to this therapy might be due to cell-to-cell contact- and humoral-mediated interactions. Further analyses of leukemic cells, host-derived immune and MSC cells in these settings will help to better understand the role of microenvironment in the mediation of response to LSD1i-based epigenetic targeted therapy.

Conflict of interest: none

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Selective Inactivation of Cancer Drugs by SAMHD1 Provides a Molecular Rationale for Therapeutic Stratification in AML

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Background: Nucleoside analog (NA) drugs are widely used to treat a variety of cancers, including acute myeloid leukemia (AML). With an essential role in regulating the cellular dNTP pool by degrading cellular nucleotides, SAMHD1 has the potential to decrease the cellular concentration of frequently prescribed NAs and thereby diminish their clinical efficacy in cancer therapy.
Method: In this study, we used biochemical, structural, and cell based methods to examine the interaction of SAMHD1 with various AML cancer drugs, including cytarabine, cladribine, clofarabine, fludarabine, gemcitabine and the two DNA-hypomethylating agents (HMAs) decitabine and azacytidine.
Results: We found that both the catalytic and the allosteric sites of SAMHD1 can bind NAs and that the SAMHD1 substrate specificity is regulated by 2′ sugar modifications of the nucleotide analogs. Cell culture, AML blasts and xenotransplantation models confirmed the crystallography findings that most of these drugs are affected by SAMHD1 activity, while some stay unaffected. In accordance with these data expression levels of SAMHD1 are correlating with survival parameters in patients treated with SAMHD1-dependent NAs.
Conclusion: Taken together, these results establish SAMHD1 as a substrate-specific resistance factor that has promise as a predictive biomarker for drug stratification and a therapeutic target in nucleoside analog-based AML therapy.
Conflict of interest: none

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A Targeted NGS Panel for Cost-Effective and Sensitive Detection of Leukemic Markers in AML Patient Samples

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Background: Acute Myeloid Leukemia (AML) is a disease with poor prognosis with about two thirds of patients relapsing after achieving complete remission (CR). Although intra-tumoral mutational heterogeneity has previously been described its emergence and course in disease progression yet remains elusive. Additionally, subclones comprising only small fractions of the leukemic cell population might carry mutational markers associated with elevated therapy resistance and adverse treatment outcome. Such subclones would ultimately be selected for by chemotherapy and cause a therapy-resistant relapse. Hence it is important to reliably detect adverse subclones not only already at the stage of diagnosis, but also in minimal residual disease (MRD) and CR stages in order to follow up the success of therapy.
Aim and method: However, due to errors introduced via library preparations and sequencing, classical Next-Generation Sequencing (NGS) approaches provide only limited sensitivity, precluding detection of mutations with variant allele frequencies (VAFs) below ~1% even with very high sequencing depths. Therefore, we are establishing a targeted NGS panel based on single-molecule Molecular Inversion Probes (smMIPs) to allow for cheap and highly-sensitive genotyping of known mutational markers in AML patients. smMIPs follow a hybridization-capture protocol for NGS library preparation to selectively enrich and amplify hundreds of gDNA target loci simultaneously in a single reaction.
Results: The incorporation of unique molecular identifiers (UMIs) within the hybridization-probes significantly increases precision of the data, allowing to reliably detect mutations with VAFs below 0.5%. Due to the high multiplexing capabilities, library preparation costs are below 5€ per sample. Moreover, as the panel consists of oligonucleotide-probes which are synthesized separately, target loci can easily be excluded, added or exchanged at any time. Hence, the NGS-panel can be adapted to include new markers or to only target a subset of the loci in order to increase the sequencing depth of the most important markers, thereby providing even higher sensitivity to detect variants in those regions. Currently we included important coding regions of DNMT3A, TET2, TP53, RUNX1 and ASXL1, as well as mutational hotspots of NPM1, IDH1, IDH2, FLT3, NRAS and KRAS amongst others in our probe pool.
Conclusion At the moment we thereby cover more than 16kb of target regions in total. In order to test the uniformity of probe-performances we collected preliminary sequencing data. Downsampling tests showed that 2.2M NGS reads per sample are enough to obtain a median unique coverage of 529x, theoretically enabling detection of mutations down to 0.2% VAF. : We are now balancing the relative probe concentrations in the pool to balance sequencing depth of the targeted gDNA loci in

order to obtain a more even sensitivity for the detection of mutations across all targets.
Conflict of interest: none

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Functional Characterization of the G372V and T454M Mutations in the Splicing Factor 1

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Background: The splicing factor 1 (SF1) recognizes the branch point sequence near the 3'splice site during the first steps of spliceosome assembly. Yet, it is not essential for constitutive splicing but implicated in alternative splicing. Somatic mutations in SF1 were identified in a subset of patients with hematological malignancies (MDS, *de novo* AML and myeloproliferative neoplasms (MPN)). Among the 5 initially described SF1 mutations, only the G372V and T454M could be found in all isoforms.
Aim and method: *In silico* studies using PolyPhene2 and MutatorTaster softwares predicted a disease causing impact of these mutations. Thus, they were chosen for the study. We searched for further *SF1* mutations in the TCGA and the COSMIC databases. Although at low frequency, *SF1* is a commonly mutated gene in a range of cancers. The mutations are widely spread within the coding region and are mainly missense, deletions and splice site mutations. The pre-RNA recognition KH-QUA2 domain is the most common target, followed by the proline-rich region, where most of the hematological-associated mutations are found, in addition to one within the N-terminal U2AF ligand motif (ULM), responsible for $U2AF^{65}$ interaction. Constructs harboring SF1-G372V and -T454M mutations were generated by site directed mutagenesis.
Results: Immunofluorescence experiments in HeLa cells demonstrated that endogenous SF1 localizes to the nucleus and overexpressed SF1 localizes in the nucleus only or both in the nucleus and cytoplasm to different extent depending on the cell examined. The same was observed for overexpressed SF1-G372V and -T454M. Of note, SF1 changes the subcellular localization of the splicing factor SRSF2. Upon SF1 overexpression, endogenous SRSF2 was partially or totally withdrawn from the nuclear speckles, to display a distinct punctate pattern within the nucleus and/or to agglomerate into granules at the cytoplasm. The same effect on SRSF2 was observed when the mutated proteins were overexpressed. In order to investigate whether SF1 regulates mRNA splicing *in vivo*, we transiently expressed increasing amounts of SF1 in HeLa cells and analyzed the splicing pattern of the cotransfected E1A minigene reporter plasmid. Increasing amounts of SF1 shifted the splicing site selection towards the most proximal 5' splice site, resulting in accumulation of the smaller 9S transcript, in a dose dependent manner. Conversely, overexpression of increasing amounts of SF1-G372V and -T454M, did not alter the splicing pattern of the E1A minigene. Thus, G372V and T454M mutations impaired the minigene splicing function of SF1, probably by interfering with protein interactions stablished by the proline-rich region of SF1. Finally, retrovirus transduced lymphoma and leukemia cell lines (Namalwa and U937) stably expressing SF1, SF1-G372V and -T454M were established and submitted to RNAseq. Comparison between gene expression pattern of the SF1 and mutated expressing cell lines are currently ongoing.
Conclusion: Taken together, our results suggest that the splicing of a subset of SF1 targeted mRNA could be impaired in leukemia patients harboring the SF1 G372V and T454M mutations. Moreover, high levels of SF1 expression could interfere with the splicing function of SRSF2. Altered SF1 function could impair splicing and be linked to leukemogenesis.
Conflict of interest: none

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Leukemogenesis in Seven Patients with Donor Cell Derived Myeloid Neoplasms. Whole Exome Sequencing Reveals Clonal Dynamics

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Background: Donor cell derived myeloid neoplasm (DCMN) is a rare complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). We report on 7 DCMN (Spanish cohort) with the objective of describing the clonal architecture and spatial heterogeneity and of identifying somatic mutations inducing in the process of leukemogenesis.
Method: A total of 32 BM samples were analyzed at different time points after allo-HSCT and one PB sample from each donor. Whole exome

sequencing (WES, SureSelect-XT) was performed in all samples.

Results: Mutational profiles obtained from the follow-up samples demonstrated high intra-tumor genetic heterogeneity and clonal dynamics. The number of variants increased over time. WES identified gene mutations commonly seen in adult AML or MDS. Other novel non-silent variants were acquired in all cases. Among the additional novel altered genes, 23 strong candidates with oncogenic potential were found. CNVs analysis revealed numerical alterations across the post allo-HSCT samples in 6 patients. The most common chromosomal alterations were chr. 7 abnormalities. Interestingly, although none of the donors developed a myeloid neoplasm at the moment of diagnosis of DCMN, one donor revealed an abnormal karyotype. All other donors harbored one pathogenic or probably-pathogenic variant, most probably of germline origin, in genes involved in hematological or solid tumor predisposition.

Conclusions: Our integrated multi-step analysis revealed intra-tumor heterogeneity and evolutionary history of 7 DCMN, showing a process of sequential clonal expansions promoted by the acquisition of somatic mutations. Detection of heritable or acquired gene mutations in donors could have clinical implications for the patients undergoing allo-HSCT. Leukemic transformation of donor stem cells provides a useful in vivo model to study the mechanisms involved in leukemogenesis.

Conflict of interest: none

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Insulin-Like Growth Factor Binding Protein 7 Activates the Retinoid Acid Differentiation Pathway in Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) is characterized by the accumulation of malignant blasts with impaired transcriptional differentiation programs. Despite important advances in AML therapy, only 30-40% of AML patients survive five years after diagnosis. This poor prognosis is mainly caused by survival of chemotherapy resistant leukemic cells, named leukemic stem cells (LSC), re-initiating relapse. However, for AML patients with PML-RARA positive acute promyelocytic leukemia (APL), treatment with all trans retinoic acid (ATRA) results in cure rates of >90%. Upon ATRA treatment, APL cells can restore transcription leading to granulocytic differentiation, and in combination with arsenic trioxide APL cells go into apoptosis. While the success of ATRA treatment has been demonstrated for APL patients, so far it has not proved effective for non-APL AML patients.

Methods and results: Previously we showed that insulin-like growth factor binding protein 7 (IGFBP7) induces apoptosis and sensitizes AML cells to chemotherapy-induced cell death. To test whether IGFBP7 may also unlock ATRA-driven differentiation, we down- or upregulated IGFBP7 levels in the APL cell line NB4. We found that IGFBP7 knockdown blocked ATRA-driven differentiation, whereas IGFBP7 overexpression increased differentiation in presence of low concentrations of ATRA, together suggesting a role for IGFBP7 in ATRA-driven differentiation in APL cells.

Furthermore, we tested the potential of IGFBP7 to induce susceptibility for ATRA-driven differentiation in primary non-APL AML cells. ATRA-IGFBP7 combination treatment showed enhanced efficiency to induce differentiation of $CD45^{dim}CD33^{+}$ AML cells and/or to reduce viability of $CD45^{dim}$ AML cells *in vitro*, and diminished the *in vivo* engraftment potential of primary AML stem and progenitor cells more effectively in NSG mice, as compared to treatment with either drug alone.

To identify factors responsible for IGFBP7-induced ATRA sensitivity, we generated gene expression profiles of primary AML treated with IGFBP7, and identified growth factor independent protein 1 (GFI1) as one of the top down-regulated genes upon IGFBP7 stimulation. As overexpression of GFI1 in non-APL AML cells led to a reduction in IGFBP7-induced susceptibility to ATRA-driven differentiation, low GFI1 expression is suggested to be associated with susceptibility to ATRA in AML cells.

Conclusions: Our results indicate that treatment of AML patients with a combination of ATRA and IGFBP7 might prevent relapse and improve AML patient survival.

Conflict of interest: none

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Long-Term Dormancy is Reversible in Patients' AML Cells Growing in Mice

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Introduction: Therapy resistance and relapse represent a major challenge for patients with acute

myeloid leukemia (AML) and are associated with dismal prognosis. This is often attributed to cells with a quiescent phenotype. Here, we identified and characterized long-term dormant cells in AML patient-derived xenografts (PDX).

Methods: Serially transplantable PDX transgenic for luciferase and a fluorochrome were analyzed. To track proliferation dynamics PDX were labeled with the proliferation sensitive dye CFSE. Cells were re-isolated at different time points after transplantation, and CFSE content analyzed by flow cytometry. Long-term dormant, label-retaining cells (LRC) were defined as cells that divided less than 3 times, while rapidly growing cells (nonLRC, nLRC) divided more than 7 times during *in vivo* growth.

Results: A rare subpopulation of LRC was detected at day 14 in 7/8 AML PDX samples, despite a general increase in tumor load. Time course analysis of 3 samples up to 29 days after transplantation revealed that dormant cells can still be detected at late time points.

To determine whether LRC and nLRC differ in their drug response, mice were injected with CFSE labeled PDX cells, received 3 doses of cytarabine and one dose of DaunoXome (days 7-9), and treatment response was analyzed at day 10. Treatment reduced tumor burden by approximately one order of magnitude compared to controls in all 4 PDX samples analyzed. Importantly, the relative proportion of LRC within all re-isolated AML cells increased after treatment, indicating that dormant LRC preferentially survive chemotherapy.

To determine whether dormancy is a constant or reversible trait, we first re-transplantated low numbers of LRC and nLRC of 2 PDX samples. This revealed similar growth behavior of all tumors. Second, we asked if nLRC are able to switch into dormancy. Therefore, high cell numbers of bulk cells and nLRC from 1st recipient mice were re-labeled with CFSE, transplanted into secondary recipients, and CFSE distribution analyzed. Transplanted nLRC and bulk cells gave rise to a similar fraction of dormant cells, indicating that nLRC are capable to switch into dormancy after re-transplantation. These data reveal a remarkable major functional plasticity of AML PDX cells in mice.

Conclusion: AML PDX contain a rare subpopulation of dormant and treatment resistant cells. Dormancy as well as reversibility of the dormant phenotype are important biological characteristics of AML cells that need to be considered when designing treatment strategies that aim to eradicate drug resistant AML cells.

Conflict of interest: none

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Loss of KDM6A Confers Drug Resistance in AML

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Background: Acute myeloid leukemia (AML) is an aggressive hematologic cancer resulting from the malignant transformation of myeloid progenitors. Despite intensive chemotherapy, relapse remains a major hurdle in the treatment of AML. Recently, we found *KDM6A* as a novel relapse-associated gene in 2/50 CN-AML patients. KDM6A (or UTX) is a histone 3 lysine 27 demethylase and a member of the COMPASS complex, important for chromatin enhancer activation.

Aim and method: In this study, we investigated the role of KDM6A during disease progression and the implications of KDM6A loss regarding drug resistance.

We found three AML patients with enrichment of *KDM6A* loss-of-function mutations at relapse and relapse-specific loss of KDM6A expression in three additional patients. In addition, in 4/8 patient-derived xenograft (PDX) samples from relapsed AML patients, KDM6A protein levels were low or completely lost.

To investigate if KDM6A loss renders cells more resistant to therapy with agents such as cytarabine (AraC), daunorubicin (DNR), or 6-thioguanine (6-TG), we silenced KDM6A expression by shRNA or CRISPR/Cas9 in K562 and MM-1 cells.

Results: Compared to control, *KDM6A* KD and KO cells showed a proliferative advantage after AraC and DNR treatment. Inducible re-expression of KDM6A in *KDM6A*-null cells suppressed proliferation and sensitized cells again to AraC treatment. To unravel the mechanism of drug resistance, we performed RNA-Seq analysis in these cells. We compared differentially expressed genes with known key candidate genes in drug metabolic pathways and found that the membrane

transporter *ENT1* was consistently downregulated in *KDM6A* KD/KO cells. Competitive inhibition of ENT1 by NBMPR lead to decreased sensitivity towards AraC suggesting that AraC resistance in *KDM6A* KO cells is conferred, at least partially, by downregulation of ENT1.
Conclusion: In conclusion, our results show that mutations in *KDM6A* are associated with the outgrowth of drug-resistant clones and highlight KDM6A as a novel marker of drug resistance in AML.
Conflict of interest: none

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Detection of Germline Mutations by Next Generation Sequencing in Myeloid Neoplasms

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Background: NGS has led to the detection of different germline alterations related to the development of neoplasms. The aim of this study is to characterize constitutional mutations associated with a genetic predisposition to hematological malignancies in patients diagnosed from myeloid neoplasms (MN).
Methods: A gene panel-based approach (MyeloidNeoplasm-GeneSGkit;
SistemasGenómicos, Spain) was carried out in 80 patients at diagnosis. The gene panel includes several genes related to hereditary MDS/AML predisposition syndromes. The discovery analysis was conducted by NGS (MiSeq; Illumina) and the germline origin of those variants with an allele frequency higher than 0.4 was confirmed by Sanger sequencing in skin fibroblasts or remission samples. When a germline variant was detected, the analysis was extended, if possible, to the patient relatives.
Results: A total of 80 patients were referred for testing. Among them, 7 cases were confirmed to harbour germline mutations in the following genes: *CEBPα, ASXL1, CSF3R, TP53, MPL* and *GATA2*. The median age at diagnosis was 41 years (range 20-73) and 71% (5/7) of the patients had onco-hematologic family background. Mutations in *CEBPα* (p.His24AlfsTer84) and *TP53* (p.Arg282Trp) have been previously described in other pedigrees. The germline origin of the *CEBPα* gene mutation was confirmed in remission samples from the patient and her mother, while that of *TP53* was confirmed on skin fibroblasts from the proband. In the case of the *ASXL1* gene, the variant found (p.Gly704Arg) has been described only as a somatic mutation. However, it has also been detected in a remission sample from the patient. The *CSF3R* variant (p.Glu835Lys) is located in a hot-spot region of the gene. Although the variant in the *MPL* gene has not been previously described, the patient presents a strong family history of thrombocytosis and mieloproliferative syndromes. The patients who carried mutations in *GATA2* (p.Arg396Gln and p.Arg396Trp) were diagnosed from MonoMAC syndrome. Both mutations were validated in skin fibroblasts.
Conclusions: The implementation of gene panel analysis by NGS in the management of patients with MN allows the identification of germline mutations. This is important not only for the patients themselves but also for their relatives, and may have an implication in donor selection for HSCT. The germline mutations found should always be confirmed on a tumour-free sample, ideally on cultured fibroblasts.
Conflict of interest: none

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Inference and Simulation of Clonal Phylogenies in Acute Myeloid Leukemia Using Single-Cell RNA-SEQ

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Introduction: Single-cell (sc) RNA-seq has emerged as a powerful tool to understand clonal heterogeneity and evolution in cancer. Unique molecular identifiers (UMIs) were introduced to deal with amplification bias, which occurs during scRNA-seq library preparation.
Aim and method: Here, we explore the potential of UMIs to proofread sequence errors for the analysis of mutations and subclonal architecture in cancer samples.
We implemented a computational pipeline to call single-nucleotide variants (SNVs) on scRNA-seq data with UMIs, and to determine the single-molecule consensus sequence of each UMI-barcoded transcript. We integrate the UMI-consensus SNVs with whole exome sequencing data to infer the subclonal architecture of the tumor. In order to test our UMI-consensus analysis pipeline, we developed a tool to simulate scRNA-seq reads with UMIs that also includes the Simulations of SNVs given a user-defined clonal architecture. We employ our simulation tool to

improve calling SNVs and estimating errors under realistic scenarios of clonal evolution.
Furthermore, we applied this pipeline on scRNA-seq data from patient-derived xenograft (PDX) samples of acute myeloid leukemia (AML).
Results: Our pipeline improved the sensitivity and reduced the false discovery rate of variant calls compared to a standard pipeline without UMI consensus.
Conclusion: In conclusion, our tools provide the opportunity to analyze clonal heterogeneity in AML at the single-cell level.
Conflict of interest: none

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Targeting miRNA-551B, a Stemness-Like Microrna, to Eradicate AML (STEM) Cells

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Background: Despite high complete remission (CR) rates achieved after chemotherapy, only 30-40% of patients with Acute Myeloid Leukemia (AML) survive five years after diagnosis. The main cause of this treatment failure and poor prognosis is the insufficient eradication of a subpopulation of chemotherapy-resistant leukemia cells with stem cell properties, named "leukemic stem cells" (LSCs). LSCs use a variety of mechanisms to resist chemotherapy and targeting them is one of the major challenges in AML treatment. Since it has been shown that miRNAs can target multiple genes/pathways simultaneously, their modulation (downregulation or upregulation) may have great potential for the successful elimination of therapy-resistant leukemic (stem) cells (*Martiañez Canales et al. Cancers 2017*). Here, we show that miRNA-551b, previously identified by us as a stem cell-like miRNA, can be a potential novel target to specifically eradicate AML stem-like cells.
Aim: To identify specific therapeutic targets in AML that can have the potential to eliminate AML LSCs.
Methods: Aiming at identification of miRNA-based therapy to specifically eradicate LSCs, while sparing normal Hematopoietic Stem Cells (HSCs), we determined microRNA expression profiles of HSCs, LSCs and leukemic progenitors (LP) all derived from the same AML bone marrow and identified miRNA-551b as being highly expressed in the HSC/LSC population. We theorized that this miRNA is specific of a stem cell population. To further elucidate the link between miRNA-551b and AML "stemness" and to test whether downregulation of miRNA-551b affects the survival of AML (stem/progenitor) cells, proliferation and the balance between differentiation and "stemness", we reduced miRNA-551b expression, either by lentiviral transduction of antagomirs or by adding locked nucleotide acid (LNA)-oligonucleotides to AML cell lines and primary AML cells and measured clonogenic capacity, proliferation and growth in vitro and in vivo. miRNA-551b targets which can be involved in the maintenance of a stem cell phenotype have also been studied.
Results: miRNA expression patterns in highly enriched HSCs, LSCs, and leukemic progenitors, all derived from the same patients' bone marrow, were determined and differentially expressed miRNAs between LSCs and HSCs and between LSCs and leukemic progenitors were identified. miRNA-551b was the top differentially expressed miRNA between residual HSCs and LSCs, showing high expression in HSCs (*de Leeuw et al. Leukemia 2016*). MiRNA-551b was not only highly expressed in residual HSCs in AML but also in HSCs residing in healthy bone marrow, suggesting a link between "stemness" and presence of miRNA-551b. Notably, AML cases with enhanced miRNA-551b expression are associated with a poorer clinical outcome than those with lower miRNA-551b expression, potentially reflecting the influence of "stemness" on therapy sensitivity.
Downregulation of miRNA-551b in the stem cell-like AML cell line KG1a led to inhibition of cell growth *in vitro*, which was due to inhibition of proliferation rather than induction of apoptosis. KG1a subcutaneously tumor growth in *an in vivo* mouse model was also reduced when miRNA-551b was downregulated. In primary AML, miRNA-551b knockdown resulted in a significant decrease in the survival of leukemic progenitors and LSCs, while hematopoietic stem cells (HSCs) and normal progenitors from healthy bone marrows were not affected. These results suggest that a therapeutic approach inhibiting miRNA-551b expression might specifically interfere the survival of long self-renewal leukemic progenitors and LSCs from primary AML, while sparing HSCs. We are also currently studying miRNA-551b targets which can be responsible for this specific LSCs elimination, and we have found FOXO3A as a possible target of miRNA-551b.
Conclusion: In conclusion, our results suggest that inhibition of miRNA-551b could be a promising approach to eliminate stem cell-like AML cells, thereby decreasing relapse rates and improving AML treatment outcome.
Conflict of interest: none

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Single-Cell RNA-Sequencing & Genotyping of AML Patients with *WT1*-Subclonal Mutations to Elucidate Clonal Heterogeneity

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Introduction:| Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic progenitor cells. After chemotherapy, most patients reach a complete remission (CR) defined by morphological criteria but ultimately, two third of the patients relapse. Only half of the relapsed patients reach a second CR, suggesting clonal evolution and possible selection of more aggressive subclones during chemotherapy. However, the mechanisms underlying resistance of certain clones are largely unknown. Therefore, it is of great relevance to gain deeper insight into the (sub)clonal diversity of AML and the biological differences between these clones. Here we aim to elucidate clonal heterogeneity on the transcriptome level in eight AML patients with subclonal *WT1* mutations using a novel single-cell RNA-sequencing method (mcSCRBseq) combined with a single-cell transcriptome genotyping approach to reveal the *WT1* mutation status in each cell.

Methods: We used cryopreserved cells from eight AML patients with subclonal *WT1* mutations (15-40% variant allele frequency, determined by targeted amplicon sequencing). Per patient, 576 single-cells were FACS-sorted while enriching for viable myeloid blasts ($CD33^{+}$, $CD45^{low}$, Annexin V, live-dead-stain). RNA-sequencing libraries were prepared according to the mcSCRBseq protocol, which includes cellular and mRNA-molecule barcoding and is very powerful and cost-effective. Libraries were sequenced at ~ 250.000 reads per cell. Single-cell genotyping was performed from full-length cDNA after the scTAGseq protocol, which allows bringing the mutation site in close proximity of the cell- and molecular barcodes by a circularization step.

Results: We will show the results of combined single-cell RNA-sequencing and per-cell genotyping for *WT1* mutation status from a total of ~4500 cells out of a cohort of eight AML patients with subclonal *WT1* mutations.

Conclusion: We aim to identify potential expression clusters with a focus on genotype-phenotype correlation and furthermore differential expression patterns between individual clusters and subclones. Differentially expressed genes might reveal regulatory pathways specifically affected by *WT1* mutations in AML.

Conflict of interest: none

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Simulating Clonal Evolution in Acute Myeloid Leukemia (AML): Bridging the Gap by Mathematical Modelling

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Background: From recent genetic studies AML pathogenesis is considered as a multistep process starting from an initial mutation in hematopoietic stem (or progenitor) cell (HSC). Clear prediagnostic data about exact timepoint and following latency period with further mutations is rare. We used mathematical modelling to simulate this biological process and potentially illustrate new insights into AML pathogenesis.

Methods: Our analysis is based on an established mathematical model (Stiehl et al., J.R.S. Interface, 2014) that uses ordinary differential equations to model hematopoiesis in a bone marrow scenario with feedback interacting stem and differentiated cells. We implemented a realistic first mutation event (via poisson process) that can arise from healthy hematopoietic stem cells during cell division over simulated time (90 years). Additionally, we added a second mutation event occurring after potential first mutation in leukemic stem cells.

Then, we varied properties (i.e. proliferation rate, self-renewal capacity, death rate) of arising leukemic clones and monitored cell trajectories of healthy and leukemic cells with leukemia diagnosis at 20% leukemic cells in the bone marrow model.

Results: With assumed low mutation rate of stem cells our model showed that first mutation type was a rare event occurring in decade intervals over simulated 90 years. Hereby, mutation can arise in childhood up to in old age (>60 years). Single increasing of the leukemic proliferation rate could not lead to sufficient leukemia development, but a slight increase in self-renewal (compared to HSC) lead to leukemia diagnosis in old age. An extreme increase in self-renewal nearly shifted diagnosis time to right after first mutation without relevant latency. In the case of slight increased self-renewal of first mutation second mutation only emerged in old age.

Conclusion: Our model results support hypothesis that AML initial (preleukemia) mutations can

emerge early in life-time and become clinical apparent in older age. Besides, results imply that self-renewal ability is at least more relevant for initial mutation in AML. In future, the model could be used to simulate clonal selection process due to treatment.
Conflict of interest: none

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The Cytogenetic Profile of 94 Patients with Acute Promyelocytic Leukemia

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Aim: The aim of the study was to identify the spectrum of chromosomal abnormalities in addition to the classic translocation t(15;17)(q24;q21), as well as variant translocations, in patients with acute promyelocytic leukemia (APL), by conventional and molecular cytogenetic analysis.
Materials and methods: Ninety four patients (51 men and 43 women) diagnosed with APL were enrolled in the study. The cytogenetic analysis was performed on 24h and 48h unstimulated bone marrow cultures using GTG-banding. Karyotypes were described according to ISCN 2016. Molecular cytogenetic analysis was performed by fluorescence in situ hybridization (FISH) especially in cases with variant t(15;17) and with additional chromosomal abnormalities to t(15;17). XL t(15;17) translocation/dual fusion probe was used to detect the chromosomal region 15q24 (PML gene), 17q21 (RARA gene) and the PML-RARA and RARA-PML fusion genes. Moreover, RARA break-apart probe was used to detect RARA variant translocations. For each sample, 200 interphases (iFISH) and 10 metaphases (mFISH) were analyzed.
Results: The sex ratio (males/females) was 1.19 and the mean age was 50 years (range 10-80). The karyotypic analysis was successful in all patients. Classic t(15;17)(q24;q21) was found in 88 patients (93.6%), in 70/94 (74.5%) as a sole abnormality and in 18/94 (19.1%) with additional chromosomal abnormalities. The most frequent additional abnormalities were trisomy 8 and ider(17)(q10) (4.3% each). Less frequent abnormalities were: add(21q)(2.1%), -7, del(11)(q21), del(9)(q22), t(8;12)(q24;q13), t(1;14)(q21;q32), del(2q), add(15)(p11.2), der(20), t(12;20)(q13;p13), del(X)(p11.1) (1.1% each) and marker chromosomes (3.2%). Variant translocations including t(15;17;19)(q24;q21;q13.1-13.3), t(15;18;17)(q24;q21;q21), t(7;17;15)(q22;q21;q24), t(X;15;17;1) (q13;q24;q21;p36), t(11;15;17) (p13;q24;q21) and t(11;17)(q23;q21) were observed in 6/94 patients (6.4%).
Conclusions: APL is characterized by a variety of chromosome abnormalities in addition to t(15;17) and different variant translocations, while the exact prognosis of each of them is not known due to their rarity. Our results underline the necessity of karyotype in the identification of the whole spectrum of chromosome abnormalities in APL and the necessity of FISH for identification of t(15;17) variants. Two new variants, t(X;15;17;1)(q13;q24;q21;p36) and t(11;15;17)(p13;q24;q21) are described for the first time in the present study.
Conflict of interest: none

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Functional Dominance of CHIP-Mutated Hematopoietic Stem Cells in Patients Undergoing Autologous Stem Cell Transplantations

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Introduction: Hematopoiesis is maintained by a polyclonal pool of hematopoietic stem cells (HSCs). Recurrent somatic mutations cause the expansion of blood cell clones in elderly persons, a phenomenon entitled clonal hematopoiesis of indeterminate potential (CHIP). However, direct evidence of the fitness of CHIP-mutated human hematopoietic stem cells (HSCs) in blood reconstitution is lacking.
Method: Since myeloablative treatments and transplantation put enforced stress on HSCs, we followed 81 patients with solid tumors or lymphoid diseases undergoing autologous stem cell transplantation (ASCT) for the development of CHIP. Using amplicon-based sequencing of 55 myeloid cancer genes, we found that 18 patients (22%) harbored CHIP with a variant allele frequency (VAF) >2% in their blood after ASCT, with a high mean VAF of 10,7%. DNMT3A was

affected most often, followed by PPM1D, TET2, , TP53, ASXL1, KDM6A, BCOR KRAS, and RAD21. To explore whether the mutations had been present before the high-dose chemotherapy, and whether the CHIP-mutated HSCs expanded after ASCT, we deeply sequenced the frozen samples of the transplanted grafts.

Results: The mutations were found in the majority of patients in the graft; however, with significantly lower VAFs than in the blood after ASCT, demonstrating a selective advantage of mutated HSCs outcompeting normal HSCs upon stress hematopoiesis. Neutrophil reconstitution was significantly delayed in patients carrying CHIP, and pronounced in patients with CHIP mutations in DNA-damage response genes. Importantly, these patients showed a trend towards a shorter survival after CHIP detection in Kaplan-Meier survival analyses.

Conclusion: We demonstrate here that CHIP-mutated HSPCs dominate in blood reconstitution after ASCT, and largely gain on clone size upon stress hematopoiesis, leading to an increased future risk of CHIP-associated complications.

Conflict of interest: none

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Developing a Model System for the Study of WILM'S Tumor 1 - Gene Mutations in Acute Myeloid Leukemia

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Background and objectives: *Wilms Tumor 1 (WT1)* gene encodes a zinc finger transcription factor with four major protein isoforms due to two major alternative splicing events. In addition, alternative translational start leads to production of further protein isoforms. These isoforms are expressed with fixed ratios throughout development in different tissues. WT1 is highly expressed in all types of human leukemias and mutated in 20% of acute myeloid leukemias (AMLs). It therefore can resume both tumor suppressor and oncogenic functions. We and others have previously shown that the ratios of these isotypes can vary between different AML subtypes. Our aim here is to develop a model system to study *WT1* and its patient-specific alterations in normal and leukemic hematopoietic cells.

Methods and results: To express *WT1* wild-type in target cells, the longest isoform of *WT1* was cloned into our vector, 5' in-frame of EGFP using the endogenous *WT1* promoter or the strong promoter of the spleen focus-forming virus (SFFV). After lentiviral transduction, *WT1* and GFP were successfully expressed in U-937, HT1080 and Hela cells (all known for their absent expression of *WT1*). Next, to express all four major isoforms using a single construct, we included the shortened introns 4, 5, and 9, which are responsible for the alternative splicing of *WT1*. Lentiviral transduction of these constructs led to expression of all four natural occurring isoforms within the previously described ratios. Furthermore, to perform functional/localization experiments, an internal ribosome entry site (IRES) was cloned in each construct between *WT1* and EGFP coding regions and to provide a selection strategy, EGFP was replaced with neomycin-resistance gene in four additional vectors. Further constructs for *WT1* knock-down and knock-out using shRNAs and CRISPR/cas9 lentiviral vectors have also been generated. Analysis of the functional effects of these tools and development of vectors expressing patient-specific *WT1* mutations in AML blasts and normal CD34+ human hematopoietic progenitor cells are currently in progress.

Conclusion: Our constructs are useful tools for functional studies of wild-type and mutated *WT1* in normal and malignant cells of any type.

Figure 1. *WT1* expression construct including alternative splice sites on introns 4, 5 and 9.

Conflict of interest: none

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IDH1 Mutation Augments System-Level Metabolic Flexibility that Favors Mitochondrial Oxidative Phosphorylation and Drug Resistance in Acute Myeloid Leukemia

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Introduction: Isocitrate dehydrogenases (IDH) are involved in redox control and central metabolism. Mutations in IDH induce epigenetic and transcriptional reprogramming, differentiation bias, BCL-2 dependence and vulnerability to mitochondrial inhibitors in cancer cells.

Results: Here we show that this mitochondrial dependency is due to increased mitochondrial metabolism including TCA cycle fluxes and oxidative phosphorylation (OxPHOS), resulting from a systemic enhancement of major catabolic fluxes in IDH1 mutant acute myeloid leukemia (AML). Mechanistically, this is mediated by an

increase in CEBPα-dependent FAO as well as higher rate for glucose, pyruvate and glutamine oxidation. Thus, multiple carbon sources are contributing to 2-HG production and other biosynthetic pathways allowing IDH mutant cells to adapt to changes in nutrient availability. This enhanced catabolism converging towards TCA cycle increases mitochondrial dependency and impact chemoresistance. Furthermore, while systematically decreasing levels of 2-HG, IDH1 mutant inhibitors do not necessarily abrogate mitochondrial metabolic capacities and OxPHOS activities in clinic.
Conclusion: Altogether these results attest to the scientific rationale of clinical trials incorporating combination therapies targeting IDH mutant activity and mitochondrial function in the presence and absence of cytarabine.
Conflict of interest: none

II. AML – THERAPY

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AML and MDS in the Population of Ukraine in Post-Chernobyl Period (1991-2016)

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Background: Recent data on LSS cohort in Japan suggest that leukemia risks for AML and MDS still remain to be significantly increased among survivors even in 60 years after exposure. Nevertheless, the systematic studies of AML and MDS in population of Ukraine in post-Chernobyl period have not been yet provided.
Aim: The aim of the study was to analyze the data on AML and MDS diagnosed in the Reference Laboratory (RL) of IEPOR in 1991-2016.
Patients and methods: The data of diagnostic examinations of 21752 patients referred to the laboratory from 20 regions of Ukraine in 1996-2016 and representing about one third of all oncohematological patients in Ukraine have been summarized and the database covering all individual data was generated. The main forms and cytological variants of hematological malignancies were diagnosed according to FAB and WHO 2008 classifications and partially revised based on archive samples according to the recent WHO 2017 classification.
Results: 4764 AML cases and 572 MDS cases were diagnosed among patients inhabiting both regions contaminated with radionuclides and non-contaminated regions of Ukraine. The trend of increasing fraction in AML cases among the total number of diagnosed hematological malignancies in patients from contaminated regions of Ukraine was followed. Moreover, within AML group as a whole, the percentage of M0, M1 and M3 variants was higher in the patients from contaminated regions within 5-9 years after Chernobyl accident. The progressive increase in relative number of MDS cases (from 0.6% in 1991 to 6.0% in 2015) diagnosed among the patients from all Ukrainian regions with decreased median age at diagnosis (59 in females and 61 in males) was evident. The MDS percentage in the patients diagnosed in RL exceeds largely the reported MDS contribution in general structure of the oncohematological pathologies in Ukraine suggesting the gross underestimation of MDS in routine diagnostic activities.
Conclusion: Analysis of the diagnostic findings and RL database revealed the increasing trend in the relative frequency of AML in regions of Ukraine contaminated with radionuclides with a shift in the distribution of AML types within 5-9 years after Chernobyl accident. The drastically increasing number of MDS patients both in contaminated and non-contaminated regions is also worth noticing. Further studies of AML and MDS in Ukrainian population including the retrospective analysis of our archived data will be useful for elucidating whether the protracted post-Chernobyl exposure to low dose radiation may affect the structure of oncohematological pathologies.
Conflict of interest: none

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Multicentre Implementation of Next Generation Sequencing to Acute Myeloid Leukemia Patients in PETHEMA Spanish Collaborative Group

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Introduction: Acute Myeloid Leukemia (AML) is a heterogeneous disease characterized for a wide spectrum of molecular alterations. Targeted next-generation sequencing (NGS) has the potential for translation into clinical practice. PETHEMA collaborative Spanish group has designed the protocol NGS-LMA based on NGS characterization of clinically relevant alterations in

AML patients. We show inter-laboratory cross-validation and results of 497 patients.

Methods: Seven PETHEMA reference laboratories established 26 AML relevant consensus genes: ***ASXL1***, *CALR, CBL,* ***CEBPA***, *DNMT3A,EZH2,* ***FLT3****,GATA2,* ***IDH1****,* ***IDH2****,JAK2, KIT,KRAS,MPL,MLL,* ***NPM1****,NRAS,PTPN11,* ***RUNX1****,SETBP1,SF3B1,SRSF2,TET2,* ***TP53****,U2AF1,* and *WT1* (bold genes were reported as clinically relevant). NGS was made with commercial or custom panels with Ion Torrent or Illumina, quality metrics criteria: minimum coverage:100x, minimum variant allele frequency (VAF):5% and uniformity>85%. Two rounds of cross-validation were made in 10 samples harbouring 35 pathogenic variants. A quality control for *FLT3*-ITD was assessed in 156 samples by NGS and capillary electrophoresis (CE). NGS was implemented in the clinical routine analysing 513 samples of 497 patients: 424 diagnoses, 32 resistances and 54 relapse.

Results: In the first round the median coverage was 2248x(range 258-3703) and the concordance for all variants was 92%. The reported VAF was similar among laboratories but we detected a higher variation in low VAF variants. In the second round, we focussed in low VAF alterations and variant reporting. In this case, median coverage was 3193x(range 342-5118). Moreover, all laboratories showed a high concordance in detection of clinically relevant mutations (89%) and its classification according to its prognostic/therapeutic value (90%). We established the cut-off 5% to report variants because 61% of discrepant results were produced when VAF was lower than <5%.

FLT3-ITD NGS validation showed a concordance of 98%. Although NGS did not detect a long *FLT3*-ITD mutation it could detect low VAF *FLT3*-ITD in 2 negative patients by CE. In our experience we have seen NGS limitation in the characterization of long *FLT3*-ITD mutations and VAF underestimation.

1146 mutations were identified in the 513 AML samples (2,23 mutations/sample). At diagnosis, the most prevalent mutated genes were *NPM1*(25%), *DNMT3A*(24%), *FLT3*(24%)[ITD; 17%, point mutations (PM) 9%] and *IDH1/2*(20%). At resistance were *FLT3*(25%)[ITD,19%;PM,6%], *DNMT3A*(22%), and *RUNX1*(19%). Regarding relapse, *FLT3*(32%)[ITD;24%;PM,9%], *DNMT3A*(24%), *NPM1*(20%) and *RUNX1*(20%) were frequently altered.

NGS has allowed the identification of clinically relevant mutations (AML clinical guidelines and/or clinical trials) in 83% of patients. Of these, 44% showed alterations in genes which are not routinely assessed by conventional techniques (15% *TP53*, 15% *RUNX1* and 14% *ASXL1*).

Conclusions: Cross-validation results show a high concordance, reproducibility and robustness of NGS and variant reporting within PETHEMA reference laboratories. Our results show the importance of NGS to detect clinically relevant genes in AML to improve patient's risk stratification and targeted therapies.

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Outcomes in Older Patients with Newly Diagnosed High-Risk/Secondary AML (sAML) who Achieve Remission with CPX-351 versus 7+3 Induction

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Introduction: CPX-351 (Vyxeos®), a dual-drug liposomal encapsulation of cytarabine (C) and daunorubicin (D) at a synergistic ratio, is approved by the EMA and FDA for adults with newly diagnosed therapy-related AML or AML with myelodysplasia-related changes.

Method: This exploratory, *post hoc* analysis of a large randomized, phase 3 study evaluated outcomes in patients (pts) aged 60-75 y with newly diagnosed, high-risk/sAML, specifically those who achieved remission following induction with CPX-351 vs 7+3 C+D chemotherapy. Pts were randomized to receive up to 2 induction cycles of CPX-351 (100 units/m^2 [C 100 mg/m^2 + D 44 mg/m^2] on Days 1, 3, and 5 [2nd induction: Days 1 and 3]) or 7+3 (C 100 mg/m^2/d continuously for 7 d [2nd induction: 5 d] + D 60 mg/m^2 on Days 1-3 [2nd induction: Days 1-2]). Pts achieving complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles. CPX-351 was associated with significantly higher rates vs 7+3 of CR+CRi (73/153 [47.7%] vs 52/156 [33.3%]; 2-sided *P*=0.016) and CR (57/153 [37.3%] vs 40/156 [25.6%]; 2-sided *P*=0.040). Baseline characteristics of pts with CR+CRi were generally balanced between arms.

Results: Median overall survival was longer with CPX-351 vs 7+3 in pts who achieved CR+CRi (25.43 vs 10.41 mo; HR=0.49 [95% CI: 0.31-0.77]) or CR (25.43 vs 10.97 mo; HR=0.49 [95% CI: 0.29-0.83]). Of pts with CR+CRi, 40/73 (55%) in the CPX-351 arm and 24/52 (46%) in the 7+3 arm underwent transplantation; median overall survival landmarked from the date of transplant was not reached vs 11.65 mo, respectively (HR=0.42 [95% CI: 0.20-0.86]). Serious AEs in ≥5% of pts with CR+CRi were febrile neutropenia

(CPX-351: 15%; 7+3: 12%), acute respiratory failure (7%; 2%), ejection fraction decreased (5%; 4%), sepsis (5%; 4%), pneumonia (3%; 6%), and pulmonary edema (1%; 6%). Four pts with CR+CRi died during the treatment phase (sepsis [CPX-351]; atrial fibrillation [7+3]; disease progression [7+3]; intracranial hemorrhage [7+3]); no pt experienced early (60 d) mortality in either arm. Median neutrophil ≥1,000/μL and platelet ≥100,000/μL recovery, respectively, in pts with CR+CRi was longer with CPX-351 (37 d and 42 d) vs 7+3 (29 d and 32 d).

Conclusion: In conclusion, CPX-351 was associated with longer survival among pts who achieved a CR or CRi, suggesting potentially deeper responses, and a safety profile consistent with the known profile of 7+3.

Conflict of interest: Stefan Faderl, Robert J. Ryan, Michael Chiarella, and Arthur C. Louis are employees of and hold stock ownership in Jazz ACL additionally has patents/royalties with Jazz. Geoffrey L. Uy has received consulting fees from Curis and GlycoMimetics. Gary J. Schiller has received research funding from Astellas Pharma and Bluebird Bio; and participated in advisory committees for Astellas Pharma. Jorge F. Cortes has received consulting fees from Jazz, Novartis, Daiichi Sankyo, Pfizer, and Astellas Pharma, and research funding from Jazz, Novartis, Daiichi, Sankyo, Pfizer, Astellas Pharma, and Arog. Ellen K. Ritchie has received consulting fees from Incyte, Celgene, Pfizer, and Novartis; research funding from Pfizer, Novartis, Astellas Pharma, Bristo-Myers Squibb, and Ns Pharma has participated in speakers bureaus for Incyte, Celgene, Novartis, an ARIAD Pharmaceuticals, and received travel support from Celgene and Novartis.

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High Aldehyde Dehydrogenase Activity at Diagnosis Predicts Poor Outcomes in Patients with T(8;21) Acute Myeloid Leukemia

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Background: Acute myeloid leukemia (AML) with t(8;21) is a heterogeneous disease, and about 40% of patients relapsed after achieving complete remission (CR). Leukemia stem cells (LSCs) is the major cause of the recurrence of AML. Some studies demonstrated that aldehyde dehydrogenase was highly expressed in leukemic stem cells and high ALDH activity ($ALDH^{high}$) predicted poor outcome in intermediate and high cytogenetic risk AML patients. However, the prognostic role of $ALDH^{high}$ in t(8;21) AML patients remains unknown.

Methods: A total of 66 t(8;21) AML patients were included. 48 of them received consolidation therapy (chemotherapy: 36, allogeneic hematologic stem cell transplantation (allo-HSCT): 12) after achieving CR by induction therapy. ALDH activity of bone marrow nucleated cells collected at diagnosis was stained using ALDEFLUOR$^{\circ,R}$ Kit (STEMCELL Technologies) and tested by flow cytometry (FCM). $CD34^{+}CD38^{-}ALDH^{high}$ cells were sorted from one patient using FCM and detected by fluorescence in situ hybridization (FISH) using specific probes for RUNX1-RUNX1T1.

Results: 52 patients were followed up with a median of 20 (1-34) months. The 2-year relapse-free survival (RFS) rate was 78.3% (95% confidence interval (CI) 57.9-89.6%). The median percentages of $CD34^{+}CD38^{-}$ and $CD34^{+}CD38^{-}ALDH^{high}$ cells were 2.6% (range 0.085-35.7%) and 0.012% (range 0.0003-0.62%), respectively. Receiver operating characteristic curve analysis showed that 4.0% and 0.24% were their individual optimal cutoff value for differentiating relapse. Patients with > 4.0% of $CD34^{+}CD38^{-}$ cells tended to having lower 2-year RFS rate than patients with ≤ 4.0% of $CD34^{+}CD38^{-}$ cells (58.2% [95% CI 21.3-82.7%] vs. 87.6% [95% CI 65.3-96.0%], p=0.078). Patients with > 0.24% of $CD34^{+}CD38^{-}ALDH^{high}$ cells had significantly lower 2-year RFS rate than patients with ≤ 0.24% of $CD34^{+}CD38^{-}ALDH^{high}$ (63.6% [95%CI 37.6-89.7%] vs. 87.8% [95% CI 71.2-100.0%], p=0.024). Similar results existed if the patients who underwent allo-HSCT were censored at the time of transplantation (p=0.065 and p=0.033). A < 3-log reduction in RUNX1-RUNX1T1 transcript levels after the second consolidation therapy was significantly related to higher percentage of $CD34^{+}CD38^{-}ALDH^{high}$ cells (0.024% [range 0.0080-0.62%] vs. 0.0085% [range 0.0006-0.080%], p=0.0057). FISH result showed that 93% of CD34+CD38-$ALDH^{high}$ cells sorted from a patient were RUNX1-RUNX1T1 positive.

Conclusion: High percentage of CD34+CD38-$ALDH^{high}$ cells at diagnosis may be relevant to relapse in t(8;21) AML patients.

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Conflict of interest: None

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Safety and Clinical Activity of Mutant IDH1 Inhibitor IVOSIDENIB (IVO; AG-120) In Combination with AZACITIDINE (AZA) for Newly Diagnosed Acute Myeloid Leukemia (ND AML)

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Background: IVO is an oral inhibitor of the mutant IDH1 (mIDH1) protein that is approved in the USA for the treatment of m*IDH1* relapsed or refractory AML. Here we report results for all patients (pts) treated with IVO + AZA from a phase 1b/2 study of pts with m*IDH1* ND AML (NCT02677922), as of 01Aug2018.
Methods: Adults with m*IDH1* ND AML ineligible for intensive treatment received IVO 500 mg orally QD + AZA 75 mg/m^2 SC on Days 1–7 in 28-day cycles. CR with partial hematologic recovery (CRh) was sponsor-derived, and defined as morphologic CR with an ANC >0.5×10^9/L *and* platelets >50×10^9/L. m*IDH1* clearance in bone marrow mononuclear cells was assessed by BEAMing Digital PCR (limit of detection 0.02–0.04%).
Results: 23 pts received IVO + AZA (11 male; median age 76 years [61–88]). Median duration of exposure was 8.4 months (0.3–22.6). 14 pts remained on treatment at time of data cut. Treatment-related adverse events (AEs) in ≥10% of pts were nausea (57%), vomiting (35%), diarrhea (26%), fatigue (26%), QTc prolongation (26%), neutropenia (22%), injection-site erythema (22%), constipation (17%), IDH differentiation syndrome (17%), thrombocytopenia (17%), and anemia (13%). Grade 3/4 AEs, regardless of causality, in ≥10% of pts were thrombocytopenia (48%), anemia (44%), febrile neutropenia (44%), neutropenia (26%), sepsis (22%), QTc prolongation (13%). CR + CRh rate was 65% (n=15), including 13 pts (57%) with CR. ORR, based on IWG criteria, was 78% (n=18), including CR (57%), CRi/CRp (13%), and MLFS (9%). Median time to response was 1.8 months (0.7–3.8), and median time to CR 3.5 months (0.8–6.0). m*IDH1* clearance was observed in 10/15 pts with CR/CRh (67%), including 9/13 (69%) with CR.
Conclusions: The combination of IVO + AZA is a well-tolerated, non-intensive strategy for pts with m*IDH1* ND AML. AEs were consistent with the safety profiles of the individual agents. Most responding pts had deep remissions based on m*IDH1* clearance.
Conflict of interest: none

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Molecular Profiling and Outcome of Patients with IDH1/2 Mutated Hematologic Malignancies after Treatment with IVOSIDENIB or ENASIDENIB

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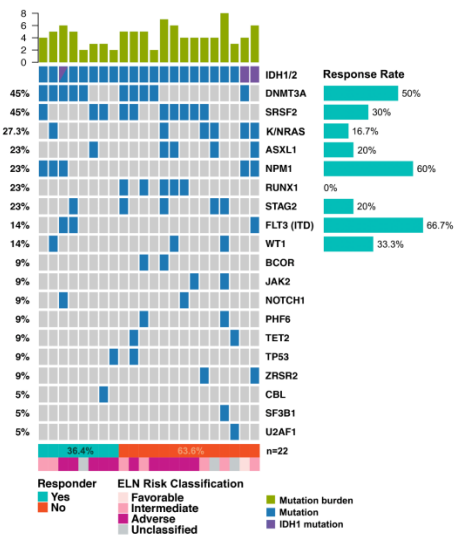
Background: Somatic mutations in isocitrate dehydrogenase (*IDH*) 1 or 2 can lead to a neomorphic enzymatic activity ultimately resulting in impairment of hematopoietic cell differentiation. Ivosidenib(IVO) and enasidenib (ENA), IDH1 and IDH2 inhibitors, respectively, were recently approved for use in patients with relapsed/refractory AML. We describe the survival outcomes and associated genomics of our cohort at Moffitt Cancer Center (MCC). **Methods:** We retrospectively analyzed a clinically and molecularly annotated cohort of 26 patients treated with IVO or ENA at MCC from 2016- 2018. Kaplan-Meier analysis with log-rank test was performed to estimate median overall survival (OS) from the time of AML diagnosis until the time of death from any cause.
Results: A total of 26 patients (12 females/14 males) were treated with either IVO (11.5%) or ENA (88.5%).Median age was 68 (range 32-83) years. Response to therapy is known in 22 patients and their molecular status at the time when the *IDH* mutation was identified is depicted in Figure 1. The overall response rate (ORR) was 36.4% (n=8) (2 complete response (CR), 3 CR with incomplete hematologic recovery (CRi), 1 CR with partial hematologic recovery (CRh), 2 partial response (PR)). The remaining 14 patients had no response (NR). Rate of *IDH1/2* clearance was 0% upon subsequent disease evaluation among responders. *IDH1/2* mutations frequently co-occured with *DMNT3A* (45%), *SRSF2*(45%), *RAS*(27.3%), *ASXL1*(23%), *NPM1* (23%), *RUNX1* (23%), and *STAG2* (23%). A trend toward higher ORR was noted in patients with co-occuring FLT3-ITD mutations (66.7%), and a significantly higher response rate was found in ENA treated patients with co-occuring NPM1 mutations (60%) (p=0.0491). On the contrary, a trend towards lower ORR of ≤20% was seen with concurrent *RUNX1, RAS, STAG2* and *ASXL1* mutations however not statistically significant. The variable allelic frequency (VAF) of *IDH1/2* mutations at the time of therapy initiation did not correlate with response. OS of the entire cohort was 38.4 months. With a median follow-up of 6.2 months from the time of IDH inhibitor initiation, the median OS was 8.3 months and median duration of therapy of 4.9 months.



Conclusion: In our series, the overall ORR of IVO and ENA is similar to that reported in the literature. VAF of an *IDH1/2* mutation did not correlate with response to IDH inhibitors. None of the co-occurring somatic mutations were identified to impact the responses to IDH inhibitors although trends were noted. Future studies to better characterize the molecular profile and its impact on clinical response are needed.
Conflict of interest: none

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Outcome of Acute Myeloid Leukemia with Inv(3)(Q21q26.2)/ T(3;3)(Q21;Q26.2). Experience of the Spanish PETHEMA and CETLAM Groups

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Introduction: Acute myeloid leukemia (AML) with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) is a rare type of poor prognosis AML recognized in the WHO 2008 classification. The aim of this study was to evaluate the outcome of patients treated according to the PETHEMA and CETLAM intensive therapy protocols and their overall survival (OS).
Methods: All patients diagnosed with *de novo* AML with inv(3)/t(3;3) treated with intensive therapy protocols and reported to PETHEMA and CETLAM groups between 1999 and 2017 were included. A total of 65 patients were identified, 61 received induction therapy according to idarubicin and cytarabine 3+7 protocols of PETHEMA and CETLAM groups and 4 patients were treated with the FLAG-IDA schedule. We retrospectively analyzed their clinico-pathological characteristics, the response rate to first line and following treatments and their OS.
Results: The median age was 45 [18:69] and 21 patients had an ECOG score ≥2. Twenty-five patients (38%) had -7/del(7q), 12 (18%) had a complex karyotype and 5 (8%) had a monosomal karyotype. Myelodysplasia was observed in 44 patients (71%). Eighteen patients (28%) achieved complete response (CR) after first induction treatment and 7 after a second line treatment (ORR: 38%). In the univariate analysis, only dysmegakaryopoiesis was associated with a higher rate of CR after first-line therapy (33% vs. 6%, p=0.039). Allogeneic stem cell transplantation (alloSCT) was performed in 38 (59%) patients, 21 (57%) were in CR and 16 (43%) had active disease. CR was observed in 28 (75%) patients after alloSCT and 11of them achieved first CR after this procedure. The median follow-up was 4.7 years [0,76 ; 10,11]. The 1-yr non relapse mortality was 24% (12%-39%). Seventeen patients relapsed after alloSCT with a 1-yr relapse incidence of 44% (27%;60%). Global 1-yr-OS was 43%, being 15% at 4 years. Patients with platelet count below 100 $x10^9$/L at diagnosis had worse OS (11.6 vs 6.9 months, p=0.034) and those with monosomal karyotype showed a trend to worse OS (11.6 vs. 8.4 months, p=0.062).
Conclusions: AML with inv(3)/t(3;3) has poor prognosis, due to low rate of CR and high relapse rate. Although AlloSCT can be useful in a subset of refractory patients, improved therapeutic strategies are clearly needed.
Conflict of interest: none

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RAS Mutations Confer an Increased Risk for Relapse in Patients With AML Receiving Induction Chemotherapy

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Background: The prognostic significance of RAS mutations in AML has been the subject of controversy. Treatment with high dose cytarabine consolidation after complete remission is associated with a greater benefit in survival in patients with RAS mutated than in RAS WT AML, suggesting a more chemosensitive disease. This study aimed to determine the prognostic impact of RAS mutations in patients receiving induction chemotherapy.
Methods: Patients with RAS mutated AML were compared to a control cohort of sequential RAS WT patients treated with induction at Memorial Sloan Kettering from January 1, 2014 to September 30, 2018. Response was evaluated per ELN 2017 response criteria, OS was calculated from time of induction to death or last follow-up, RFS was calculated from time of response with induction to relapse, death or last follow up. Baseline characteristics were evaluated by χ^2 analysis and Student t tests. Kaplan-Meier estimates were used to summarize OS and RFS.
Results: A total of 44 patients with RAS-mutated AML were included in the analysis and compared to 83 patients with RAS WT AML. Baseline characteristics for the RAS mutated population included a median age of 62 years, male sex in 55%, prior MDS in 39%, a therapy related myeloid neoplasm in 18%, prior treatment with a hypomethylating agent in 18%, a median bone marrow blast percentage of 39% at diagnosis and an ELN 2017 risk stratification of 23% favorable, 32% intermediate, 45% adverse risk. There was no significant difference in baseline characteristics between RAS and WT groups. Both RAS mutated and RAS WT patients were treated with standard cytarabine and anthracycline based induction chemotherapy regimens. Patients requiring reinduction were similarly treated preferentially with an intermediate or high dose cytarabine containing regimen. There was no difference in response between RAS mutated and RAS WT patients receiving only one induction regimen (CR MRD negative rate 39% vs. 33%, p= 0.79, CR+ CRi: 69% vs. 68%, p =0.94). Re-induction led to significantly increased responses in RAS mutated patients (CR+CRi 67% vs. 27% p<0.01). Both groups had a similar percentage of responders undergoing allogeneic transplantation (70% vs. 74% p=0.7). RAS mutations were associated with a trend towards decreased overall survival and a significantly decreased relapse free survival after treatment with induction chemotherapy (OS: 25.2 months vs. 32.2 months, p=0.07; RFS: 25.3 months vs. 36.8 months, p=0.04).
Conclusions: Our data suggest that RAS mutant AML may not be as chemosensitive as previously thought. Although RAS mutated and RAS WT AML have similar response rates to induction chemotherapy, RAS mutations conferred a shorter relapse free survival and a trend towards decreased survival.
Conflict of interest: none

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Do Frailty Scores Help Defining Fitness of Elderly AML Patients at Diagnosis? A Single Centre Study

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Background: Treatment of elderly acute myeloid leukemia (AML) patients represents a challenge due to both, more aggressive disease biology as well as patient-related risk factors. Several studies demonstrated improved survival receiving intensive induction chemotherapy. But defining those patients ”fit” for intensive chemotherapy is challenging and would require objective and validated criteria. The aim of the present study was to investigate the power of three validation scores in assessing patient fitness at diagnosis in parallel to physician evaluation. Further patient outcome according to the respective assessment was compared.
Methods: A total of 130 elderly (≥60 years) newly diagnosed AML patients were treated. Initial haematologist evaluation followed by discussion of the patient case in an interdisciplinary board lead to decision of therapy intensity. In parallel, independently from the medical board decision, three scores were performed: i) a local geriatric G8 screening tool, consisting of seven items from the Mini Nutritional Assessment questionnaire and age, ii) the Sorror Index used for hematopoietic stem cell transplantation (HSCT) evaluation and iii) the AML score proposed by the German AML Cooperative Group, predicting probability of complete remission (CR) and early death (ED) after intensive induction chemotherapy. Therapy response was defined according to ELN criteria. Overall survival (OS) from diagnosis was compared between groups using the Cox model.
Results: Median age was 71,8 years (range 60-86) A total of 75 (57,7%) patients were evaluated “fit” by the medical board and treated by intensive chemotherapy (“7+3” regimen), whereas 41 (31,5%) underwent semi-intensive therapy and 14 (10,7%) received best supportive care. Fifty patients (38,5%) achieved a CR after induction chemotherapy, ten of them underwent allogeneic HSCT. Sixty-four (49,2%) were non responders and 16 patients (12,3%) died during the first cycle. Overall, the median survival time was 11,2 months (95% CI 7,9-17,8). Primary physician care

evaluation was able to define a "fit" from an "unfit" patient. Median survival time from "fit" patients was 17,6 months (95%CI 9-28,9) compared to "unfit" evaluated patients with 3,6 months (95%CI 1,8-8,8), p<0.001 with a HR (unfit vs. fit) of 3,04 (95% CI of 1,80-5,15). The local G8 screening tool was able to distinguish significantly "fit" patients with a median OS of 18,7 months from "unfit" patients with a median OS of 7,9 months, HR (unfit vs. fit) 2,1 (95% CI 1,23-3,58), p=0,007. The Sorror Index (HR 2,14 with a 95% CI of 1,27-3,59) as well as the AML score ED (HR 1,94 with 95% CI of 1,16-3,24) separated also significantly "fit" from "unfit" patients, p=0,004 and p=0,011, respectively. There was no correlation between the scores detected as investigated by the Spearman Correlation coefficient.

Conclusion: In conclusion, the frailty scores G8, Sorror Index and the AML Score discriminate patients quite well in terms of OS. They may represent an additional tool in the validation of frailty in elderly AML at diagnosis. In order to validate the discrimination ability arising from the present performed analysis, a multi-centre study is planned.

Conflict of interest: none

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Response to Front and Second Line Treatment in Patients with Acute Non Lymphoblastic Leukemia: A Single Center Experience

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Background: Acute non- lymphoblastic(ANLL)is the type of leukemia with the worst prognosis. Over the past 30-years treatment of ANLL consisted of two successive phases. The first or frontline therapy aimed to achieve leukemic cell clearance from the bone marrow, while the second line therapy is mainly to prevent relapse. This study assessed response to front and second line therapy in patients with ANLL.

Methods: Data of 90- patients with ANLL were retrospectively collected from hospital records of those who were admitted and treated at the Department of Medical Oncology at South Egypt Cancer Institute(SECI) in the period 2000-2010. Ethical approval by the research ethical committee at SECI was a prerequisite before data collection. Multiple hospital records for single patient were gathered and a full disease history was obtained with particular stress on response to treatment, incidence of relapse and date of death. The collected data were coded and analyzed by (SPSS/PC/VER 17. Patients were treated with two frontline regimens, adriamycin and non-adriamycin containing regimens. Second line therapy was in the form of HAM (33.9%), consolidation for M3 (6.5%),(59.7%) of our patients discontinue treatment. Those who were planned for HSCT were referred to other center as this facility was unavailable at SECI at the time of the study.

Results: The mean age was (37.9 ± 6.9 years), 51.1% were males. The ECOG status was 1 in 84.4 % & CNS infiltration in 5.6%. M2, M3 followed by M4 were the commonest FAB subtypes. 68.9% of patients achieved complete remission (CR), however rate of remission was different according to different induction courses. Patients who received HAM, 17 patients continue remission for a period ranging from 3 to 18 month till bone marrow transplantation. While patients who didn't received HAM, 11 patients suffered from relapse (p value = 0.5349).

The longest overall (OS)& disease free survival(DFS) were 20& 18 month in 10.6% & 1.1% respectively. Again survival analysis was different with different courses of induction. Those who achieved CR after first 1st induction had longer OS& DFS, p =0.5.

Conclusion: In conclusion the current study provided a scientific evidence that early blast clearance is a good prognostic factor in ANLL. Also it encouraged post remission therapy with HAM to lengthen the relapse free period till HSCT. However a big problem with HAM is the patient incompliance, accordingly great effort from hematologists has to be exerted to keep their patients in regular treatment.

Keywords: ANLL, frontline therapy, second line.

Conflict of interest: None

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Prognosing Efficacy of FLAG Regimen in theTreatment of Relapsed and Refractory AML

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Aims: Evaluation of the FLAG/FLAG-Ida regimen efficacy, identification of the factors that influence the achievement of complete remission (CR) and duration of the relapse-free and overall survival (RFS, OS) in patients with relapsed/refractory AML.
Methods: We analyzed 54 adult patients (28 males and 26 females, median age 37 years; range 18-70), 27 patients (50 %) were refractory, 27 patients (50 %) were in relapse. FLAG/FLAG-Ida regimen was used as induction therapy. 37 pts (68,5 %) were underwent alloHSCT. Molecular genetic, cytogenetic studies were conducted before therapy and on the 28th day of therapy. WT1 gene expression was also assessed on the 14th day of induction.
Results: 77,8 % patients (42/54) achieved CR, 16,7 % (9/54) were refractory, there were 5,5 % (3/54) of early death. The frequency of CR was higher in relapsed than in refractory AML (85,2 % (23/27) and 70.4 % (19/27), respectively). Achievement of CR did not depend on the time of relapse, risk group (ELN) (90,9 % (10/11), 83,3 % (20/24) and 78,9 % (15/19), $p > 0,05$). Patients with blast level at day 14 ≥ 10 % had a significantly lower rates CR (60 % vs. 89,6 %, $p = 0,024$) and shorter RFS (median 7,6 vs. 17,6 months, respectively, $p = 0,03$). RFS was shorter in patients with WT1 reduction less than 1 log at 14 day (median 5 vs. 18 months, respectively, $p = 0,01$). In the group of patients with blast level < 10 % at 14 day the duration of RFS depended on the WT1 level (median 6,3 vs. median not reached, respectively, $p = 0,04$). Thus, the WT1 level is a more sensitive marker of the duration of RFS than blast level. Patients with MRD-negative status (57,1 %) had significantly longer RFS, OS (median RFS 17,6 vs 5,2 months, $p = 0,02$; median OS 19 vs 6,9 months, $p = 0,0002$). The duration of RFS and OS differed between favorable and unfavorable risk groups (ELN): median not reached vs 5,2 months, $p = 0,039$, median not reached vs 10,2 months, $p = 0,039$, respectively. The duration of RFS and OS of intermediate risk group (median 7,6 months vs 13,05 months, respectively) had no significant differences from other groups.
Conclusions: The FLAG/FLAG-Ida regimen was shown to be effective in relapsed/refractory AML. The achievement of CR did not depend on the risk group, the time of relapse. The level blasts at 14 day is prognostic factor that influence the achievement and duration of CR. The WT1 level in the early post-induction period is sensitive marker of duration of the RFS. The MRD level and molecular-genetic group risk (ELN) are important prognostic factors of the duration of RFS and OS.
Conflict of interest: None

Decitabine as Salvage Therapy after Azacitidine in AML

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Introduction: Hypomethylating agents (HMA) are the reference treatment for AML not candidates for intensive chemotherapy (*Kantarjian et al JCO 2012, Dombret et al Blood 2015).* The outcome for AML patients who relapse to HMA is dismal, median OS 2 months *(Nanah R et al Am J Hematol. 2017).* The MDA-AML-2017-05 study analyzed 228 AML patients treated in Spain with decitabine *(de la Fuente et al EHA 2018).*
Aim: The aim of this study is to analyze effectiveness and tolerance of Decitabine (Dec) as salvage therapy after Azacytidine in AML in day to day practice and to identify risk factors.
Methods: We carried out the analysis on AML patients previously treated with Aza included in the MDA-AML-2017-05 study on 23 Spanish sites. Inclusion criteria as follows Age >18, diagnosis of AML under WHO criteria, treated with Dec during the period 01/09/2014 to 31/12/2016. We evaluated effectiveness as ELN-2010 criteria, toxicity as CTCAE v3.0 scale, OS by Kaplan-Meier and the mortality within the first 8 weeks (M8wks). This study has been approved by the Spanish Medicines Agency AEMPS code MDA-AML-2017-05.
Results: Of the 228 patients included in the MDA-AML-2017-05 study 102 received Dec as salvage therapy, of them, 22 (15V, 7M) received Dec after Aza. Average age 76 (58-87), 80 yrs and above 7p, ECOG≥2: 9p, Creatinine>1.3 mg/dL: 4p, adverse cytogenetic: 4p, WBC pre-Dec >15.000/μL: 9p. A total of 73 cycles were analyzed, median 3 (1-10), at the end of follow up 17 patients died, no cases of treatment related mortality. Seventeen pts were evaluated for effectiveness ORR35.2% (CR1p, PR5p) and ED2p. The M8wks was 27 % and

median OS 5 months (1yr OS 15%). Among patients treated with Dec as salvage therapy (102p) previous Aza resulted in statistical differences for OS (9 vs 5 ms p0.04). In this analysis WBC pre-Dec >15.000/μL (p <0.04) and ECOG≥2 (p<0.01) resulted in statistical differences for OS. Age >80 (p0.9), creatinine >1.3 mg/dL (p0.5), adverse cytogenetics (p<0.4) revealed no differences.

Conclusions: In our experience response rates with Dec after Aza were modest. Even the OS seems superior to previously reported it is inferior to 6 months.

	HR	95,0% CI	P
Reduction *NPM1*4lg *RUNX1-RUNX1T1* 2,4lg	10,55	1,6 - 69,9	0,02
Aberant karyotype	2,5	0,5 - 11,4	0,25
Postinduction reduction *WT1* 2log	0,12	0,2 - 6,8	0,91
FLT3ITD, *CKIT mutation*	2,99	0,7 -12,9	0,14

Conflict of interest: none

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MRD Level after Induction Therapy in *NPM1* Mutation and *RUNX1-RUNX1T1* Positive AML Identifies High Risk of Relapse Patients

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Aims: To evaluate prognostic significance of MRD levels after 1st and consequent cycles of therapy in pts with *RUNX1-RUNX1T1* and *NPM1*mut.

Patients and methods: 17 pts with NPM1mut and 17 pts with *RUNX1-RUNX1T1* were evaluated in the study. Additional abnormalities were revealed in 14 pts (MLL (#1), *FLT3ITD* (#7), *DNMT(#1)*, *C-KIT* (#1), aberrant karyotype (#5)). All pts had *WT1* overexpression. «7+3» was used for remission induction and «HiDAC» for consolidation. 15 pts underwent alloHSCT.

Results: 94,1% (32/34) pts reached AML remission. 37,5 % (12/32) had relapse (3/12 after HSCT). Pts with *FLT3-ITD* are characterized by poorer RFS (median was 4,6mo vs not reached, p=0,012). A cut-off ratio for RFS after induction therapy for *NPM1*mut was 0,015% for absolute ratios and 4,0lg for a kinetics of reduction. Median RFS was not reached vs 3,0 mo, p=0,026 for absolute levels, and median was not reached vs 2,6 mo, p=0,006 for kinetics. Both cut-off levels had the high sensitivities (100%) and specificities (60% and 70%). Both cut-off levels *RUNX1-RUNX1T1* (1,5% for absolute ratios and 2,4lg for a reduction) with the sensitivities 100% and specificities 60% identified pts with a short RFS as well (median was not reached vs 6,3 mo, p=0,001 for absolute levels and kinetics). 15/32 pts with CR showed MRD levels above the estimated cut-off after induction. 9/15 pts were treated with «HiDAC» for consolidation, without HSCT and had further reduction MRD levels, even so 9/9 pts had relapse in the next 12 mo. In one out of the 6 pts *NPM1*mut wasn't detected at relapse, but at the same time level of *WT1* persisted elevated. The cut-off level for RFS of *WT1* reduction was 2 lg after induction. The level of reduction for more than 2 lg prognosed longer RFS (p=0,00006). This favorable group is subdivided by reduction level of *NPM1* and *RUNX1-RUNX1T1* less 4 and 2,4 lg respectively in this group had adverse impact (4,6 mo vs median was not reached, p=0,005). In multivariate analysis (included *FLT3ITD, CKIT*, adverse karyotype and *WT1* level of reduction) with forward selection, both *NPM1* and *RUNX1-RUNX1T1* postinduction level (p=0,02) were independent prognostic factors for RFS (**Tab**.)

Conclusions: *NPM1mut* and *RUNX1-RUNX1T1* positive pts has adverse prognosis if the postinduction reduction of expression of these genes is higher than our estimated cut-off . MRD level after induction seems to be more predictive than MRD after next cycles of therapy.

Conflict of interest: None

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Highly Sensitive Residual Disease Detection in Acute Myeloid Leukemia Using Advanced Error Corrected DNA Sequencing

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Background: Next-generation sequencing (NGS) of leukemia-specific somatic mutations represents a potentially attractive solution for measurable residual disease (MRD) estimation in acute myeloid leukemia (AML) patients but accuracy for variants present at low levels is limited by technical constraints.

Aim and method: To benchmark the performance characteristics of potential solutions we tested conventional NGS, single-strand error-corrected consensus sequencing (SSCS) and duplex sequencing (DS) on an AML patient sample diluted into normal donor DNA at ratios from 1/100 (1%) to 1/10,000 (0.01%). The AML sample tested contained mutations in *CBL, DNMT3A, NPM1, RAD21* and *FLT3*. NGS libraries were each run on 2 different sequencers (HiSeq 2500 and NextSeq).

Results: With conventional hybrid-capture NGS sequencing the background frequency per sample

averaged $1.7x10^{-3}$ but with numerous individual sites above 10^{-2}, therefore obscuring those mutations below approximately 1%. Uncorrected background was similar across both sequencing platforms. SSCS had mean background frequencies as low as $2.5x10^{-5}$ and $1.5x10^{-4}$ per sample between the two sequencers, suggesting technical biases in certain regions due to sequencing chemistry. DS background was as low as $6.14x10^{-7}$ and $5.7x10^{-7}$ per sample on the 2 sequencers, demonstrating duplex error correction of issues such as early-round PCR errors, DNA damage or strand-specific biases from sequence context. DS detected expected mutations as low as $1.9x10^{-5}$ (ie: MAF of 0.0019%), with excellent correlation between observed and expected frequencies across the range of the dilution series using either sequencing platform (r^2 of 0.96 and 0.92). Without error correction, all base positions queried had background signal. With DS only 2.1% of sites had any "background", this was consistent across both sequencers, and was highest in *CBL* and *DNMT3A* (both associated with clonal hematopoesis and hence potentially true biological variation rather than technical noise).

Conclusion: A DS DNA-sequencing panel targeting 29 genes often mutated in AML has been developed which would, if combined with the RNA-sequencing based AML MRD assay recently reported by the NIH (PMID: 30171026), detect at least one target in most patients with AML. DS sequencing of AML mutations appears to result in substantially lower background error-rates than conventional or single strand error correction NGS approaches and may have utility in AML MRD particularly when a diagnostic sample is unavailable and/or when exceptionally high sensitivity monitoring is indicated.

Conflict of interest: none

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Sparing Anti-Bacterial Prophylaxis in Acute Myeloid Leukemia during Post Induction Aplasia: Results of a Retrospective Single Center Study

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Background: Acute myeloid leukemia (AML) patients are at high risk of infections in post chemotherapy aplasia, especially after induction. The anti-bacterial prophylactic approach raised concerns about the emergency of chemo-resistance and the increased incidence of Gram negative bacteriaemiae after consolidation.

Aim: Aim of the study was to evaluate the role of anti-bacterial prophylaxis during induction.

Methods: Retrospective single center study enrolling all consecutively newly diagnosed AML adult patients (acute promyelocytic leukemia excluded) treated with intensive standard induction therapy. Patients were divided in two groups based on anti-bacterial prophylaxis (levofloxacin 500 mg OD until neutrophil recovery or until intravenous antibiotic treatment was needed) given (group A) or not (group B). The primary endpoint was the number of bacteriaemiae. Chi-square test was used to analyze categorical variables while Student's t-test was used for continuous variables.

Results: 402 AML patients were enrolled from June 2001 to May 2018; 223 were male while 179 were female, the median age was 54 years (19-76). After induction a complete remission was achieved in 280 pts (70%), 96 were resistant (24%) and 26 died (6%). Anti-bacterial prophylaxis was given in 343 patients (group A, June 2001 - December 2016); 59 patients did not receive prophylaxis (group B, January 2017 - May 2018). Baseline characteristics were balanced between groups, except from age (median of 55 vs 62 years in group A vs B, respectively $p<0.0001$). Fever was experienced in 313 patients in group A (91%) and 56 (95%) in group B, ($p=0.3439$). The median number of fever days was similar in both groups (median 6 days, range 0-42 vs median 5, range 0-17, in group A and B, respectively, $p=0.0873$). The incidence of bacteriemiae was 25% (n=87) in group A compared to 32% (n=19) in group B ($p=0.2708$). A gram-negative was detected in 32% of bacteriemiae (n=28) vs 47% (n=9), wherease a gram-positive was detected in 68% (n=59) vs 53% (n=10) in group A vs B, respectively ($p=0.2084$). Klebsiella pneumonia carbapenemase-producing (KPC) positive blood cultures were detected in two patients in group A (one with KPC positive rectal swab), and in one in group B (whit rectal swab negative for KPC). A septic shock was evidenced in 5% (n=17) vs 11% (n=6) of patients in group A and B respectively ($p=0.1320$). Early induction deaths were similar in both groups with 22 deaths in group A (6%) and 4 in group B (7%), respectively ($p=0.9160$). In group A, we observed 80 (23%) pneumonia, 53 (15%) respiratory failure and 35 (10%) neutropenic colitis; in group B 17 (29%) pneumonia, 17 (29%) respiratory failure and 18 (29%) neutropenic colitis.

Conclusions: In our study the omission of levofloxacin prophylaxis did not cause an increase incidence of bacteriaemiae in a homogeneous cohort of AML patients. Neither fever incidence nor duration, septic shocks, early induction deaths and the rate of Gram-negative and Gram-positive cultures were different between the two groups. Our results underline the safety of a prophylaxis sparing approach that may reduce the emergence of drug-resistance Gram-negatives during the consolidation phases. Prospective studies are needed to confirm these data and to assess the impact of prophylaxis during consolidation phase.

Conflict of interest: none

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A Mathematical Model for Relapse Prediction in AML Patients Based on Continuing NPM1 Measurements

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Introduction: Acute myeloid leukemia is a rapidly progressing disease with high relapse rates. It is important to assess the therapy response as soon as possible after treatment start to decide about further treatment strategies. For $NPM1^{mut}$ patients the $NPM1^{mut}$ level in bone marrow aspirates are used for this purpose and also serve as a reliable marker for measurable residual disease (MRD) monitoring and early relapse detection. A quantitative understanding of the remission dynamics in terms of mechanistic mathematical models can further educate treatment decisions and contribute to relapses predictions.

Method: We developed a mathematical model describing the stem cell compartment of AML patients, considering competitive growth advantages of leukemic cells, stem cell quiescence, and a selective kill of proliferative cells by chemotherapy to simulate the course of AML. Time course data on $NPM1^{mut}$/Abl ratios of 256 patients were available for model validation.

Results: Adapting the model to the available data, we can reproduce the major characteristics of most patients. Specifically, we identified the leukemic growth rate as a major determinant of the time of relapse. Our model suggests, that this feature can already be evaluated in the inter-treatment phases during induction therapy. Therefore, we suggest a closer monitoring of patients during these phases as well as improved precision of measurements in order to evaluate the potential for future relapse prediction.

Conclusion: In conclusion, our modeling suggests that overall leukemia aggressiveness, as one major predictor of relapse, can already be estimated during induction therapy. Appropriate trial design will support the validation of this hypothesis.

Conflict of interest: None

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A Mathematical Approach to Assess Duration of Neutropenia within Different Induction Regimens in Acute Myeloid Leukemia (AML)

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Background: One major complication leading to increased morbidity and mortality in AML is chemotherapy-related neutropenia with severe infections. Mathematical disease modelling is a powerful approach to compare neutropenia time within different treatment regimens from a pure mechanistic point of view. With high correlation of infection risk with neutropenia duration, there is a strong motivation to find optimal strategy (e.g. treatment intensity and timing) to minimize time of neutropenia.

Methods: We modified an established mathematical model (Stiehl et al., J.R.S. Interface, 2014) that uses ordinary differential equations to model leukemogenesis and following induction chemotherapy (implemented as proliferation-sensitive and/or insensitive) in a single-major-clone-setting. We modelled standard 7+3 and variations (differing especially in treatment timing) with S-HAM and a timed sequential chemotherapy (TSC) to record subsequent cytoreduction in a standard patient for three leukemia types (fast, intermediate, slow growing). In our analysis we examine neutropenia as a model state with < 1500 neutrophils/µl and simulate different treatment intensities (from low to high with 10,201 doses) and monitor cases of complete remission (model definition: <5% leukemic cells) and occurring neutropenia duration (in days).

Results: Our modelling results show that very slow growing AML was not treatable to CR. In relevant cases of CR (dependent on treatment intensity) means of neutropenia duration for fast AML were 53.5 days (7+3), 54.3 days (S-HAM) and 59.7 days (TSC). For intermediate AML means shifted to 62.6 days (7+3), 65.1 days (S-HAM) and 67.1 days (TSC). In all cases higher treatment intensity was positively linear correlated with longer neutropenia duration, but only with significant high coefficient of determination in fast AML (0.98 for 7+3, 0.75 for S-HAM, 0.61 for TSC).

Conclusion: Concerning shortest neutropenia time 7+3 regimen showed superiority in all cases. In fast growing AML scenario S-HAM enabled at least similar results, but not for slower growing AML. With highest correlation between treatment intensity and neutropenia in case of fast growing AML, our model underlines importance of dose control in such clinical situations with aggressive (=fast growing) leukemia.

Conflict of interest: none

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Combined Inhibition of the MENIN-MLL Chromatin Complex and FLT3 Acts Synergistically against FLT3 Mutant Leukemias

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Background: The MENIN (MEN1)-MLL1 (KMT2A) chromatin complex controls leukemogenic gene expression and represents a therapeutic target in leukemias driven by MLL-fusion (MLL-f) proteins.

Results: We recently demonstrated that the interaction of menin and MLL1 is a dependency in leukemias harboring a mutation in the *NPM1* gene (*NPM1*^{mut}). Concomitant activating mutations in the gene encoding the receptor tyrosine kinase FLT3 are commonly found in both leukemia subtypes (*NPM1*^{mut}: 60%; MLL-f: 10%). RNA sequencing of *NPM1*^{mut} OCI-AML3 and MLL-f driven MV411 cells upon treatment with MI503, a small molecule menin-MLL1 inhibitor revealed the MEIS1 transcription factor and its putative target gene *FLT3* to be among the most significantly downregulated genes. *MEIS1* and *FLT3* were also downregulated in other MLL-f or *NPM1*^{mut} driven human and murine leukemias following treatment with MI503.

Allele specific qPCR for wildtype and mutant FLT3 confirmed that both alleles are profoundly suppressed in MLL-f driven MV411 and MOLM13 and murine *Npm1*$^{mut/+}$*Flt3*$^{ITD/+}$ cells. FLT3 protein expression was also substantially reduced upon MI503 treatment as assessed by FACS. Combined menin-MLL1 and FLT3 inhibition with MI503 and AC220 a second generation FLT3 inhibitor showed synergistic inhibition of proliferation and induction of apoptosis compared to single drug treatment or vehicle alone in MOLM13 and MV411 cells. HL60 and NB4 AML cells lacking an *NPM1*^{mut}, MLL-f, or *FLT3*-ITD were unaffected.

Drug synergy was also observed in the murine *Npm1*$^{mut/+}$*Flt3*$^{ITD/+}$ AML cells when combining MI503 with ponatinib a tyrosine kinase inhibitor with activity against the *FLT3*-ITD F692L resistance mutation that has been described in these cells. Of interest, ectopic expression of *Hoxa9-Meis1* resulted in *Flt3* upregulation and rescued the antiproliferative effect of combined menin- and FLT3-inhibition.

Combination treatment reduced FLT3 phosphorylation more than AC220 or MI503 alone most likely reflecting the joint effect of AC220 mediated inhibition of FLT3 phosphorylation and transcriptional *FLT3* suppression via MI503. Transcriptional profiling revealed substantial silencing of FLT3 downstream signature genes including *MYC* upon combinatorial treatment. Further assessment of primary *NPM1*^{mut} *FLT3*-ITD AML patient samples treated in a human stromal cell co-culture model showed superior inhibition of cell proliferation for the combination treatment compared to single drug treatment or vehicle alone. Finally, *in vivo* treatment of leukemic MV411 xenograft mice showed significantly reduced leukemic cell burden in animals treated with the drug combination compared to MI503, AC220, or vehicle.

Conclusion: In summary our data show that combined pharmacological inhibition of the menin-MLL1 interaction and FLT3 may represent a novel therapeutic approach to synergistically target MLL-f and *NPM1*^{mut} driven leukemias with concomitant *FLT3*-ITD.

Conflict of interest: None

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Targeting CBP/β-Catenin in MDS/AML

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Introduction: Myelodysplastic Syndrome (MDS) is often considered a pre-leukemia in that approximately one third of MDS patients progress to AML. Our previous studies have validated that highly specific small molecule CBP/catenin antagonists ICG-001 can safely and efficaciously eliminate primary patient-derived chronic myeloid leukemia and pre-B cell acute lymphoblastic leukemia by switching CBP/catenin to p300/catenin driven transcription. Therefore, we hypothesize that disruption of the CBP/catenin interaction in MDS, thereby increasing p300/catenin transcription can force symmetric differentiative divisions thus preventing progression to AML, a disease which still has a very poor prognosis.

Methods: In the present study, we have used MDS patient samples obtained from the City of Hope Liquid Tumor Banking Core: A.556, relapsed as AML post allogeneic HCT, responded to re-

induction chemotherapy and underwent second allogeneic HCT but died with multi-organ failure and A.1117 relapsed after allogenic HCT and progressed to AML and died. These patient MDS cells were used for in vitro studies after ICG-001 treatment including flow cytometric analysis, CFU and co-immunoprecipitation (Co-IP) assays and Western Blot analysis.

Results: After just 24h treatment with ICG-001 (A.556), there was a significant reduction in the expression of $CD34^+$, $CD33^+$ and $CD13^+$. Decreased expression of CD13 on monocytes is associated with favorable outcome in MDS patients. Importantly, treatment with ICG-001 led to a significant reduction of self-renewal as determined in vitro by CFU assay. A Co-IP assay showed that ICG-001 lead to a reduction in the CBP/β-catenin interaction with increase in the p300/β-catenin interaction at 24h. We further observed in these patient samples that ICG-001 down-regulated the Wnt/CBP/catenin regulated anti-apoptotic gene *Survivin/BIRC5*.

Conclusion: Our data proposes a novel MDS/AML therapy by inhibition of CBP/β-Catenin in MDS cells to prevent progression to AML.

Conflict of interest: none

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Extracellular ATP and CD39 Regulates Mitochondrial Function and Cytarabine Resistance through Intrinsic PKA-PGC1α Pathway in Acute Myeloid Leukemia

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Background: Relapses in acute myeloid leukemia (AML) are caused by chemo resistant leukemic populations and new therapeutic approaches that specifically target these cells are urgently needed. Based on transcriptomic analyses of relevant PDX preclinical model of the resistance to Cytarabine (AraC) and of the residual disease in patients, we identified ecto-nucleoside triphosphate diphosphohydrolase-1 CD39 (ENTPD1) overexpressed in the residual leukemic cells *in vivo* after chemotherapy.

Method and results: By flow cytometry, we confirmed that AraC increased cell surface CD39 expression in AML cell lines *in vitro* and *in vivo* as well as in 24 diverse patient-derived xenograft models. We further observed this increase in 100 patients at 35-days post-intensive chemotherapy compared to their respective diagnosis. Interestingly, higher CD39 ratio increase in patients was associated with a reduced event free survival in clinics. Furthermore, we showed that $CD39^{high}$ primary AML cells had increased mitochondrial mass and activity, and that FACS-sorted $CD39^{high}$ AML cells were resistant to AraC *in vitro* and *in vivo*. We demonstrated that CD39 downstream signaling pathway was dependent on cAMP-PKA-PGC1α axis and its inhibition by H89 sensitized AML cells to AraC through the inhibition of mitochondrial OxPHOS biogenesis and function. Finally, pharmacological inhibition of CD39 ATP hydrolase activity or genetic invalidation of CD39 protein using two inhibitors or shRNA, respectively, markedly enhanced AraC cytotoxicity in AML cell lines and primary patient samples *in vitro* and *in vivo*.

Conclusion: Together, these results indicate CD39 as a new player of the intrinsic chemo resistance pathway and a new therapeutic target to specifically overcome AraC resistance and eradicate these leukemic cells responsible for relapses in AML.

Conflict of interest: none

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Adrenomedullin Receptor CALCRL Drives Drug Resistance of Leukemic Stem Cells in Acute Myeloid Leukemia Extracellular ATP and CD39 Regulates Mitochondrial Function and Cytarabine Resistance through Intrinsic PKA-PGC1α Pathway in Acute Myeloid Leukemia

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Introduction: After intensive chemotherapy, the emergence of cells with drug resistant and/or stem cell features might explain frequent relapses and the poor outcome of patients with acute myeloid leukemia (AML).

Method and results: Herein, we first uncovered that the adrenomedullin receptor CALCRL is overexpressed in AML patients compared to normal cells and preferentially in the immature CD34+CD38- compartment. Then, using Limiting Dilution Assay (LDA), we demonstrated its role in the maintenance of leukemic stem cell function *in vivo*. Moreover, CALCRL depletion strongly affected leukemic growth in xenograft models and sensitized to chemotherapeutic agent cytarabine *in vivo*. Accordingly, we showed that ADM-CALCRL axis drove BCL2 pathway, cell cycle and DNA integrity in E2F1-dependent manner, and high OxPHOS status that we previously described as a feature of minimal residual disease after chemotherapy (Farge *et al.*, 2017). Furthermore, CALCRL expression predicted the response to chemotherapy *in vivo* in mice (n=10 Patient Derived Xenografts) and in patients. Further, using

the combination of Limiting Dilution Assays, single cell RNA-seq analyses of primary AML samples at diagnosis and relapse and before and after transplantation in NSG mice, we revealed the pre-existence of a chemoresistant leukemic stem cell sub-population harboring a CALCRL gene signature. Finally, we treated with cytarabine xenografted-mice and we depleted CALCRL before reinjection and LDA analysis. Therefore, we demonstrated that this receptor is required to maintain LSC-function after chemotherapy. **Conclusion:** All of these data highlight the critical role of CALCRL in stem cell function and metabolism. They also identify this receptor as a new druggable marker of chemoresistant leukemic stem cell population and a promising therapeutic target to specifically eradicate them and overcome relapse in AML.
Conflict of interest: none

III. ALL - BIOLOGY

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Impact of Exchange Transfusion for Prevention of Early Death in a Pediatric APL Patient with Hyperleukocytosis

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Introduction: The risk of early hemorrhagic death (ED) is still very high in pediatric patients with acute promyelocytic leukemia (APL) and hyperleukocytosis (WBC >10x10^9/l) and increases further with increased WBC. Several procedures to avoid severe hemorrhages are proposed, however, exchange transfusion (ET) is generally not recommended, because release of procoagulants may trigger disseminated intravascular coagulation. We present a boy with APL and rapid increase in blast counts and coagulopathy, who could be saved by emergency strategies including ET.
Case Report: The 9-year old boy - previously well - presented with pronounced hematomas on the leg and recent epistaxis. Clinical examination at admission revealed a reduced general condition, hematomas and petechiae, hepatosplenomegaly, fever of 39.4°C. Initial blood values showed: WBC 64.1x10^9/l with 58% blasts, hemoglobin 41 g/l, erythrocytes 1.4 tera/l, platelet count 12 x10^9/l. Coagulation parameters were markedly altered: prothrombin time 48%, fibrinogen <0.3 g/l, D-dimer 24.43 µg/ml. LDH was high 373 U/l. Bone marrow aspiration showed M3 with Auer bundles, FLT3 positive.
Treatment: Initially (day 1), the child was hydrogenated and got packed erythrocytes, platelets and fibrinogen. On day 2, when the diagnosis was confirmed, the clinical condition worsened with low oxygen saturation of 88%. WBC increased up to 91x10^9/l. Platelets and fresh frozen plasma (FFP) were given. ET with 4.5 liter blood was performed via Sheldon catheter over 10 hours (body volume x2 = 4.9 l). ET had to be stopped ahead of time due to recurrent and severe transfusion reactions. Cytarabine (100mg/m²/24 hours) was given during ET with an hourly bolus equivalent to the dose per hour. ATRA, dexamethasone and calcium were administered in parallel. At the end of ET the blast count decreased to 30x10^9/l, but remained still high (36-47x10^9/l) for 36 hours. After continued treatment with cytarabine and addional idarubicin (reduced dose of 6mg/m²) on day 3, the WBC count decreased to 5x10^9/l on day 4. The cytarabine/idarubicin induction was continued during the next 6 days (full idarubicin dose 12mg/m²/d x1). Coagulation normalized slowly within the next 4 days. FFP was given 7 times until day 9, 4x platelets, 2x packed erythrocytes, hydroxyurea day 3-8. Further APL treatment with ATRA and ATO (starting day 3) was administered. There were no further complications except an exanthema and the boy could be discharged after 4 weeks in good health condition. He is now after 3 months in molecular remission.
Discussion: In non-APL patients with hyperleukocytosis (WBC >100x10^9/l) ET or leukapheresis is an option to prevent ED by hemorrhage. In APL patients, the risk for ED is already high at WBC >10x10^9/l and it is recommended to give ATRA immediately together with cytostatic drugs like cytarabine and in case of coagulopathy and bleeding symptoms FFP, fibrinogen and/or cryoprecipitate and platelet transfusions. However, there are exceptional cases, as our patient, when the time period for the measures to be effective is too short. The most rapid and save method seems to be ET, which can replace the blasts with normal blood cells and also the coagulation stimulating factors in the blood plasma with normal plasma.
Conclusion: In conclusion, our successful strategy with ET in a patient with very high risk of ED by hemorrhage, might be an example for similar situations in other APL patients.
Conflict of interest: None

III. ALL – BIOLOGY

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Prognostic Importance of *IKZF1* Gene Deletions in Patients with Acute Lymphoblastic Leukemia

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Background: The Ikaros transcription factor is important for the aspects of the hematopoiesis. IKZF1 deletions have been implicated in teenagers and young adults with acute lymphoblastic leukemia (ALL). IKZF1 deletions are associated with increase in
the incidence of relapse.
Purpose: The purpose of the study was to investigate of the prognostic significance of *IKZF1* gene deletions in teenagers and young adults with ALL.
Patients and methods: We assessed in 54 patients (pts) (10.9-27.3 years) with ALL according to the clinical trial (protocol ALL-MB-2008). To detect *IKZF1* aberrations we used RQ-PCR to quantify short Ikaros isoforms expression and PCR amplification followed by sequencing to assess deletions of exons 1-6 and exons 3-6. *IKZF1* deletions has been found in 8 (14.8%) pts: 4 teenagers and 4 young adults. 46 (85.2%) pts had «wild type» (wt). 7 (87.5%) of 8 pts with *IKZF1* deletions had primary precursor B-lineage ALL and 1 (12.5%) – T-ALL.
Results: 8 (100%)/8 pts with *IKZF1* deletions achieved complete remission and 43 (93.5%) /46 pts with wt. CCR had 2 (25 %)/8 pts with *IKZF1* deletions and 28 (60.9%)/46 pts with wt, p=0.0595.
1 (12.5%)/8 pts with *IKZF1* deletions have died from infectious complications (remission death) and 4(8.6%)/46 pts with wt (early death – 2 and remission death – 2). One pts with wt hasn't responded to induction therapy. 5 (62.5%)/8 pts with *IKZF1*deletions developed relapse, 13 (28.3%)/46 pts with wt p=0.0579.Pts with *IKZF1*deletions had significantly lower EFS than pts with wt (25%±15% vs 61%±7%, p=0.0264), while cumulative incidence of relapse was higher (62.5%±19.7% vs 28.3%±6.7%, p= p=0.0267).
Conclusion: our results suggest that the increase of relapses was in pts with *IKZF1* deletions.
Conflict of interest: None

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Drawing in AF15Q14/CASC5 and APOBEC3A/C in Leukemogenesis

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Introduction: Originally, interaction between the both proteins, such as AF15q14/CASC5 and Phorbolin1/A3A/C was found in human B-cell lymphoblast line- derived cDNA library. As one of the outer kinetochore proteins, CASC5 is impicated in accurate attachment of spindle microtubules to the centromeres of sister chromatids which segregate to different daughter cells in mitosis. As a cytosine deaminase, A3A/C is involved in editing events in mammalian cells and can mediate high mutagenicity of tumor cells due to C to U conversion in centromere and inhibited function of UNG2 in the same region as was shown in CMLbc (K562).
Purpose: Determine mRNA expression levels of the both genes (CASC5, A3A/C) in bone marrow (BM) of the patients with different blood malignancies and estimate their contribution in leukemogenesis.
Methods and results: Extracted RNA samples of primary leukemia patients (n=33) were applied for RT-qPCR. The average expression levels of CASC5 and A3A/C genes were increased remarkably in BM of patients with AML[10] (n=10) and regressed in the following sequences: CML[5], APL[4], B-ALL[11], T-ALL[3]. The decreased level of CASC5 and elevated number of cytogenetic and/or molecular genetic abnormalities were associated with each other regardless of the diagnosis (AML[3], CML[2], APL[2], B-ALL[2], T-ALL[1]). The downregulated CASC5 (A3A/C) level was accompanied by simultaneous accumulation of following mutations: aml1-eto and c-kit/flt3-itd in AML[3], del(6)(q24)/del(7)(q22) and overexpression of BAALC in AML[1]; detection of alternatively spliced genes: bcr-abl (p210/p190) in CML[2], pml-rara (bcr1/2/3) in APL[1]. The lowest expression level of CASC5 (A3A/C) was coincided with observation of bcr-abl/p190 mutation (0.053%) in T-ALL[1] and correlated with high resistance to the therapy and short survival. Besides, the downregulated CASC5 and upregulated A3A/C levels were accompanied by increased expressions of tel-aml1 in B-ALL[5] or pml-rara (bcr1/2/3) in APL[1]. In the most cases, upregulated CASC5 (A3A/C) levels were matched with overexpression of WT1 in all studied types of leukemia excluding B-ALL.
Conclusion: The correlation between the gene expression (CASC5, A3A/C) and number of blast cells was observed in all studied leukemias with level of significance ($p<0.05$) in T-ALL. Besides, disregulated expressions of the both genes are resulted in growing cytogenetic and/or molecular genetic alternations including the enhansed level of WT1 (BAALC). Thus, CASC5 and A3A/C genes are involved in abnormal cell proliferation and leukemogenesis. Whether, modulating the expressions of above-mentioned targets could eliminate leukemia development will be an interesting area of future investigations.
Conflict of interest: None

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Inducible Re-Expression of KLF4 Impairs Growth of Patient Derived Acute Lymphoma Leukemia Cells *IN VIVO* and Sensitizes them towards Chemotherapy

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Introduction: Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor which acts either as oncogene or as tumor suppressor in a tissue-dependent manner. We recently found that KLF4 expression is down-regulated in primary patients' acute lymphoblastic leukemia (ALL) cells at diagnosis and even more pronounced after treatment, suggesting a tumor suppressive function of KLF4 in ALL (Cancer Cell 2016).

Aim: Here, we aimed at deciphering the role of decreased KFL4 for growth behavior and chemosensitivity in patient-derived xenograft (PDX) ALL cells *in vivo*.

Method: We transplanted primary ALL patients' sample into immune-compromised NSG mice to generate PDX cells. Lentiviral transduction allowed genetic engineering to re-introduce wildtype (wt) KLF4 and a zinc-finger DNA binding domain truncated form of KLF4 (mut). As wtKLF4 might impair ALL growth and despite major technical challenges, we established a tetracycline inducible expression system in PDX ALL cells. Coupling the transgenes to a fluorochromic marker gene and optimizing concentrations of Doxycycline (DOX) *in vivo*, we were able to re-express KLF4 at physiological levels in an on and off manner.

Results: Upon re-expression of wt, but not mutKLF4, spontaneous proliferation of PDX ALL cells was significantly diminished in two different ALL patient samples *in vivo*. Re-expression of KLF4 caused cell cycle arrest and induced apoptosis signaling including Caspase-3 and PARP cleavage. To investigate the effect of re-expressed KLF4 on chemosensitivity, mice were treated both with DOX and additionally with the conventional chemotherapeutic drug Vincristine (VCR). KLF4-expressing cells are eliminated by VCR with much higher efficiency than control cells suggesting that chemosensitivity for VCR was significantly increased upon re-expressing KLF4.

Conclusion: Taken together, our data show that re-expression of wt KLF4 to physiological levels impairs growth of PDX ALL cells in mice and sensitizes cells towards treatment. We conclude that drugs increasing KLF4 levels such as APTO-253 (Aptose Biosciences) should be developed towards clinical use for the benefit of patients with ALL, especially at minimal residual disease.

Conflict of interest: None

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Combined Inhibition of MEK-Signaling and BCL-2 Promotes Synergistic Effects in NRAS-Mutated BCP-ALL Cells

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Introduction: Acute lymphoblastic leukemia (ALL) is a genetically heterogeneous disease that originates from the malignant transformation of lymphoid progenitors of B-cell lineage (BCP-ALL) or T-cell lineage (T-ALL). This project aims to determine the sensitivity of ALL cell lines and primary cells with heterogeneous genetic backgrounds to a candidate drug library directed to specific genomic alterations in order to identify drug resistant phenotypes and rescue their sensitivity applying drug combination strategies.

Methods: Response to a target-specific library of drugs (n=40) was assessed by measuring viability/proliferation of BCP- and T-ALL cell lines and primary leukemic blasts with the WST assay after incubation for 48h with at least 4 concentrations of each drugs to obtain half-maximal inhibitory drug concentrations (IC-50; μM). Analysis of the drug combination effects of serial dilutions was carried out with Combenefit Software and validated with AnnexinV/PI staining followed by FACS analysis.

Results: We designed a drug library targeting genomic alterations in BCP-ALL, which were identified by gene panel sequencing and RNA-Seq of adult BCP-ALL samples (n=133) to guide the prediction of drug targets with potential clinical use. Application of this library to a panel of BCP- and T-ALL cell lines indicated heterogeneous sensitivity patterns. Venetoclax, targeting mitochondrial apoptosis via BCL-2 inhibition, was one of the drugs with the most variable response profile, which could in part be correlated to the BCL-2 mRNA expression. 697 and Nalm6 BCP-ALL cells showed a comparatively low sensitivity to Venetoclax (IC50 0.14 μM and 0.41 μM each) when compared to other BCP-ALL cell lines (median IC50 0.047 μM). The MEK inhibitor Selumetinib, targeting aberrant MEK-ERK activation downstream of RAS mutations showed only minimal activity independent of the cell line RAS mutation status (over 20 μM).

The combination of Venetoclax and Selumetinib markedly reduced the viability of 697 BCP-ALL cells harboring an activating NRAS mutation (p.G12D; over 65% increase of cell death compared with the sum of both single treatments). Combination effect analysis based on a Loewe model identified this combination as synergistic across a large concentration range tested. This synergistic effect was also seen in Nalm6 cells, which present NRAS A146T mutation, but not in non-mutant NRAS cell lines (SEM and REH).
Conclusions: We have established a pipeline for high-throughput-testing of targeted treatment approaches and combinations in ALL cell lines and primary samples, providing the basis for in-depth analysis of genotype-phenotype correlations.
Conflict of interest: none

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Heterogenous Activity and Syngergistic Interaction of BH3-MIMETICS VENETOCLAX, S63845 & A1331852 in B-Cell Precursor Acute Lymphoblastic Leukemia

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Background: Deregulated cell death and survival pathways contribute to leukemogenesis and treatment failure of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) patients. Anti-apoptotic proteins like BCL2, MCL1 & BCL-XL have been shown to play a key role in survival of leukemic cells. Recently, small molecule inhibitors have been developed to target those proteins. The BCL2-specific inhibitor venetoclax (VEN) has been approved for the treatment of CLL and has shown promising results in other preclinical studies including BCP-ALL. However, we and others observed variable sensitivities in individual samples, indicating that ALL cells may also depend on other anti-apoptotic proteins such as MCL-1 or BCL-XL.
Aim and method: Here, we investigated the activities of BH3-mimetics targeting BCL-2, MCL1 and BCL-XL, aiming to identify alternative treatment strategies and potential combination therapies.
The activities of VEN, the MCL1-Inhibitor S63845 (S) and the BCL-XL Inhibitor A1331852 (A) were analyzed in BCP-ALL cell lines (N=7) and patient derived xenografts (PDXs) (N=22) by cell viability assays and estimation of half-maximal effective concentrations (EC_{50}). Combinatorial effects were analyzed using the Chou-Talalay method. Functional analyses of apoptosis regulators were performed using BH3-Profiling.
Results: Single drug EC_{50} evaluation showed heterogeneous sensitivities for all three inhibitors in BCP-ALL cell lines and similarly in PDXs. Aiming to identify strategies for VEN insensitive leukemias, we evaluated the effects of A and S in these samples. Interestingly, among the VEN resistant samples (EC_{50} > 1μM; N=11) we identified 1 sample being sensitive to S and 4 samples being sensitive to A (both EC_{50} < 200nM), indicating anti-leukemia activity by alternative targeting of MCL-1 or BCL-XL in ALL not responding to VEN.
Next, we analyzed the dependence of ALL cell lines to anti-apoptotic BCL-2 family members upon exposure to VEN by dynamic BH3-profiling and identified increased dependence on BCL-XL and MCL-1 in all cell lines tested. Importantly, if combining treatment of VEN with S or A, we identified synergistic effects (CIs < 0.3) with both inhibitors in all cell lines and also in VEN insensitive PDX tested (N=5).
Conclusion: Taken together, we found heterogeneous activities of the BH3-mimetics VEN, S63845 and A1331852 in BCP-ALL. Dual targeting of BCL-2 and MCL-1 or BCL-XL resulted in synergistic cell death induction, providing an effective strategy to treat VEN insensitive ALL.
Conflict of interest: none

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RNAi-Screen Identifies Mediators of Stroma-Induced Resistance to NOTCH and mTOR-Inhibition in T-CELL Acute Lymphoblastic Leukemia

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Background: Molecular hallmarks of T-ALL are the activation of NOTCH signaling and high activity of the PI3K-AKT-mTOR pathway, constituting potential therapeutic targets. However, the in vivo performance of mTOR and Notch inhibitors in T-ALL has yielded mixed results.
Aim and method: To investigate the impact of mTOR or Notch inhibition in T-ALL we developed an aggressive murine T-ALL model. In vitro, T-ALL blasts were highly sensitive to inhibition of AKT, mTOR and Notch signaling. Upon transplantation into secondary recipients treatment with Rapamycin prolonged survival (placebo: 27 days, Rapamycin 49 days, $p<0.001$), but eventually all Rapamycin-treated animals succumbed to leukemia despite continuous drug

administration, showing infiltration of the bone marrow and solid organs, but low peripheral blast counts. Importantly, when Rapamycin-resistant blasts were isolated and cultured in suspension culture they again became susceptible to Rapamycin, demonstrating a context-dependent resistance rather than outgrowth of intrinsically resistant clones.

Results: We found an upregulation of networks associated with cell-cell interactions in Rapamycin-resistant T-ALL in vivo. Stromal cell support strikingly recapitulated the *in vivo* effect and induced robust resistance to mTOR and Notch-inhibition, which was dependent on direct cell-cell interactions. In order to find pathways that mediate stroma-induced resistance to Rapamycin, we conducted a retroviral shRNA screen interrogating genes implicated in organization of cell-cell contacts. This screen showed depletion of shRNAs targeting the IPP-complex upstream of the Rac pathway and the Rac2, but not Rac1 GTPase. We confirmed our results using independent shRNAs and Clostridium difficile toxin B (TcdBF), that selectively glucosylates and inactivates Rac-GTPases. We show that depletion of Rac2, but not of Rac1, abrogates the stroma-mediated resistance to mTOR and Notch inhibition in T-ALL without affecting the viability of T-ALL cells grown in suspension culture.

Conclusion: Altogether, we identify the Rac-GTPases as a nexus of stroma-induced drug resistance and show that inhibition of Rac and mTOR- or Notch-signaling is synthetically lethal to T-ALL blasts T-ALL blasts that are in contact with stromal cells, paving the way to augment the effectiveness of small molecule inhibitors in T-ALL.

Conflict of interest: none

IV. ALL – THERAPY

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Trends in Survival of Young Adult Patients with Acute Lymphoblastic Leukemia (ALL) in Sweden and USA

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Purpose. Several study groups have reported that introduction of pediatric-inspired therapy for young adults with ALL has improved patient survival. The Swedish ALL group embraced such a strategy in January 2009, preceded by a pilot study. Similarly, introduction of imatinib for patients with Philadelphia-positive ALL has led to additional improvements. In a population-based setting we compared the survival patterns of adult ALL patients in Sweden and the US Surveillance, Epidemiology and End Results (SEER) database with emphasis on temporal trends**.**

Methods: We identified patients diagnosed with ALL during the years 1980-2015 in Sweden (n=1,562) using the nationwide Swedish Cancer Register and in USA (n=5,124) using the SEER 9 database. The 9 states in the SEER 9 database covered 9.4% of the US population in 2010. Patients were followed up for death until the end of 2015 (USA) and 2017 (Sweden). The same selection criteria were applied for both countries. In particular, we used "international rules" for classifying multiple primaries. Relative survival ratios (RSRs) were computed as measures of net survival, thus providing measures of excess mortality associated with a diagnosis of cancer without relying on classification of cause of death. We estimated RS using flexible parametric relative survival models. For the main analysis, we focused on temporal trends in 1-year and 5-year RS for patients in age groups 18-29 and 30-44 years.

Results: Starting in the mid-1990s there was a gradual increase in 5-year RS of patients aged 30-44 years in both cohorts; 5-year RSRs were predicted to be 0.67 (95% Confidence Interval; CI 0.54-0.77; Sweden) and 0.60 (CI 0.51-0.68; SEER) in patients diagnosed in 2015. The corresponding 5-year RSRs in patients diagnosed in 1990 were 0.26 (CI 0.20-0.33) and 0.25 (CI 0.21-0.28). The steepest increase in RS was observed in Swedish patients diagnosed 2005-2015. In 18-29 year-old patients the improvement in 5-year RS started 5-10 years earlier and increased faster in Swedish patients, with 5-year RSRs predicted to be 0.71 (CI 0.56-0.81; Sweden) and 0.62 (CI 0.53-0.70; SEER) in patients diagnosed 2015, respectively. A similar but not so clear pattern was observed regarding 1-year RS for both age groups. Smaller improvements in RS were observed in age groups 45-64 and 65-84 years diagnosed in the most recent decade.

Conclusion: We believe that these results confirm, importantly also in a population-based setting, that pediatric-inspired regimens administered in young adult (<45 years of age) ALL patients improve survival.

Conflict of interest: None

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Young Adults with Acute Lymphoblastic Leukemia: Therapy Optimization in the Republic of Belarus

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Purpose: The purpose of the study was to assess results of the therapy (modified pediatric protocol) of the young adults with ALL.
Patients and methods: Protocol ALL-MB-Minsk 2010 (modified pediatric ALL-MB-2008) was used in the treatment of 54 young adults (YA) (18-29 years, mediana 22,2 yrs) with acute lymphoblastic leukemia (ALL) from January 2010. 39/72.2% patients (pts) had with B-precursor (non-T-immunophenotype) ALL and 15/27.8% – T-ALL. 46 (85.1%) of 54 YA were stratificated to intermediate risk groups and 8/14.9% – high risk group. Response on day 8 of induction therapy: 42/77.7% pts had the blast cell level in peripheral blood <1000 («good» response) and 12/22.3% – ≥1000 («poor» response). M1-status (blast cells level in bone marrow <5%) on day 15 of induction therapy had 45/83.4% YA and M2+M3-status (blast cells level in bone marrow >5%) – 9/16.6% YA. Morphological remission was attained by day 36 in 49 (90.7%) pts.
Results: Event-free survival (EFS) was 57%±7%, cumulative incidence (CI) of relapse was 31.9%±6.5%. CCR had 31/57.4% pts. 5/9.3% YA have died from infectious complications: early death – 2/3.7% and remission death – 3/5.6%. One (1.9%) YA with T-ALL hasn't responded to induction therapy. Young adults with non-T-ALL had significantly lower EFS than pts with T-ALL (46%±8% vs 87%±9%, p=0.0196), while CI of relapse was higher (43.9%±8.2% vs 0%±0%, p=0.0032). 42/77.7% with «good» response at 8 day had significantly higher EFS than pts with «poor» response (64%±7% vs 33%±14% p=0.0354); CI Relapse was 26.7%±7% (YA with «good» response) vs YA with «poor» response - 50%±15.6%, p=0.0996.
Response on day 15: EFS was 64%±7% (pts with M1-status) and 22%±14% (pts with M2+M3-status), p=0.0083. CI Relapse was significantly lower at YA with M1-status (26.7%±6.8% vs 55.6%±18.8%, p=0.0885)
Conclusion: Treatment results ALL (pediatric protocol) were better in young adults (EFS - 57%). Non-T-ALL of young adults was significantly prognostic factor.
Conflict of interest: None

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Non-Intensive but Non-Interruptive Treatment without High-Dose Blocks is an Effective Strategy for Adult PH-Negative Acute Lymphoblastic Leukemia: The First Interim Results of the Russian Prospective Multicenter RALL-2016 Study

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Background: RALL-2009 study (NCT01193933) has demonstrated that non-intensive but non-interruptive treatment with fever allo-HSCT is rather effective in adult Ph-negative ALL producing more than 50% OS at 10 years. Since Dec 2016 were started new protocol RALL-2016 that based on results of previously protocol.
Aim. To evaluate the first interim results of MRD monitoring and 1-year relapse probability (RP) regarding MRD in Ph-negative ALL patients treated by RALL-2016.
Materials and patients: Taking in consideration the major pitfalls of RALL-2009 we developed a new protocol. One day HD MTX and HD ARA-C blocks were eliminated and substituted by 2 months of non-interruptive therapy, L-asparaginase was scheduled for 1 y of treatment instead of 2,5y, 15 intrathecal injections were increased up to 21, CR T-ALL pts were brought to randomization after the informed consent: autoHSCT vs no autoHSCT, with the similar further maintenance. All included pts were MRD monitored by FCM in a centralized lab in 3 time-points (70^{th}, 133th and 190^{th} days). Since Dec 2016 till Nov 2018 91 ALL pts from 11 centers were included in RALL-2016 protocol: med age 33 y (18-54), m/f 58/33. BCP-ALL - in 49 (54%) pts, T-ALL/LBL-39(43%), biphenotypic-3(3%)).
Results: CR rate in 91 pts was 81% (n=74), induction death 10% (n=9), resistance in 9% (n=8). No death in CR. Allo-HSCT was performed in 2 (2%) of 91 pts. 30 pts with T-ALL after CR achievement were randomized for CT or autoHSCT: 15 in both groups. So far 9 of 15 T-ALL pts were transplanted at a median time of 6 m of CR. Totally 3(4%) pts relapsed (all with T-ALL). At 20m OS was 70,5%. OS and RP in T-ALL and BCP-ALL: 78% vs 62,2% (p=0,19) and 19,9% vs 0% (p=0,003), respectively. MRD positive status on $+70^{th}$ day in 16 (25%) of 64 pts (5 T-ALL and 11 BCP-ALL), on +133th day – in 9 (16%) of 58 pts (2 T-ALL and 7 BCP-ALL), on $+190^{th}$ day – 2 (5%) of 40 pts (all with BCP-ALL). We didn't registered differences in RP in accordance with MRD (pos vs neg) on all points. RP after induction were 7,7% vs 8,3% (p=0.6).
Conclusion: Our data demonstrate that the treatment approach of the RALL-2016 is rather effective and results comparable with results in previously study. MRD was detected more often in B-ALL pts than T-ALL pts. But MRD-positive status didn't significantly predict relapse in the first year of follow-up.
Conflict of interest: None

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Monitoring Measurable Residual/Relapsing Disease after Allogeneic Hematopoietic Stem Cell Transplantation in Adult Patients with Acute Lymphoblastic Leukemia

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Background: Relapse after allogeneic hematopoietic stem cell transplantation (HSCT) is a major cause of death in adult patients with acute lymphoblastic leukemia (ALL). Molecular methods to detect measurable residual/relapsing disease (MRD) significantly improved the sensitivity to trace impending hematologic relapse, but the best strategy for ALL MRD monitoring post-HSCT has yet to be defined.

Patients and Method: Here, we compared the sensitivity of MRD monitoring by mixed and CD34-sorted bone marrow donor cell chimerism (mixed and CD34+ DCC) versus quantitative PCR (qPCR) in a cohort of 57 adult ALL patients receiving a first HSCT at our institution between 09/2004 and 12/2015. An impending relapse was considered if DCC was ≤95% and/or if the ratio of rearrangement-specific/control-gene qPCR was $\geq 1x10^{-4}$.

Results After a median follow-up of 3.5 years after HSCT combined MRD monitoring by CD34+ DCC and/or qPCR turned positive in 20/57 ALL patients and (pre-)detected 12/12 hematologic relapses. MRD positivity preceded the hematologic relapse by >15 days in 7/12 cases. 17/20 MRD positive patients received preemptive or salvage therapy (withdrawal/reduction of immunosuppressive medication, chemo- or targeted therapy, donor lymphocyte infusion, second HSCT) and three patients never relapsed on a watch-and-wait strategy. 9/20 patients were alive and in complete hematologic remission (CR) at 3.5 years of follow-up, while eight patients died in relapse and three died in CR. Among the 20 MRD positive patients, a hematologic relapse occurred in 10/14 patients with CD34+ DCC and qPCR both indicating MRD, in 1/5 patients with an isolated decrease of the CD34+ DCC, and in 1/1 patient with a positive signal from T-cell receptor gamma rearrangement-specific qPCR only. Thus, the sensitivity to (pre-)detect a hematologic relapse was 92% (11/12) for both, CD34+ DCC, missing one mainly extramedullary T-ALL relapse, and qPCR, missing one case where the BCR/ABL fusion-transcript targeted for MRD monitoring could not be detected in the relapsing clone. MRD analyses by mixed DCC showed lower sensitivity (64%), with mixed DCC levels decreasing simultaneously to CD34+ DCC in 5 and at a later time in 2/11 evaluable relapsing patients.

Conclusion: In summary, these data show that both, qPCR and CD34+ DCC, but not mixed DCC, are sensitive indicators for hematologic relapse of ALL after HSCT. With the limitations of a retrospective study, our data suggest that ALL patients after allogeneic HSCT may benefit from MRD-guided therapy to avert hematologic relapse.

Conflict of interest: None

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Next Generation Sequencing as the Way for IGH Earragementmrd Monitoring in B-ALL

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Introduction: Acute lymphoblastic leukaemia (ALL) is the most common form of haematological cancer in children but it also affects adults. The clinical course of ALL is highly variable. Determination of the mutational status of rearranged immunoglobulin heavy chain variable (*IGHV*) genes in large series of patients ALL has shown powerful and independent prognostic value. Detection of minimal residual disease (MRD) after chemotherapy is the main predictor of the outcome in ALL. Next generation sequencing technology (NGS) enables through deep sequencing of rearranged IgVH CDR3 regions analysis of a previously inaccessible level of BCR repertoire. The CDR3 diversity reflects clonal composition, the potential antigenic recognition spectrum, and quantity of the available B cell responses. The aim of our study is using NGS profiling to follow up minimal residual disease (MRD) in samples from our ALL patients.

Patients and method: We have been monitoring MRD of the IgH rearrangement of 24 patients, follow up up to 3 years. The samples from the patients were collected according to their treatment protocol (age 22-72yrs, median 61 yrs, sex17 M/ 7 F).

The bone marrow samples were used for Ficoll-Paque density gradient centrifugation to obtained mononuclear cells. For *IGHV* analysis was used genomic DNA (gDNA). First step was fragment analysis with Biomed2 primers for clonality testing. Samples for NGS were amplified in tetraplicates with Biomed2[1] primers (FR1 and FR2) containing adapters for Multiplicom MIDs.Prepared libraries were analyzed using

paired-end Illumina MiSeq sequencing. Raw data were processed by our own pipeline and on-line Vidjil software (http://www.vidjil.org/). The sensitivity of this method is given by correlation between input gDNA and sequencing read depth.
Conclusion: In this study, we tried to obtain the sensitivity of sequencing greater than 10^{-4}. In conclusion, NGS showed the potential to be a very sensitive method for MRD monitoring. Furthermore, within this monitoring we can detect a new potential pathologic clone. The interpretation of results seems to be very complicated. There is need to discussed how to set up the sensitivity threshold. The other question for discussion is how to handle with observed discrepant results within FR1 and FR2. Extended analysis of a larger cohort of patients should answer these questions.
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Conflict of interest: Supported by MH CZ- DRO (UHHK, 00179906)

V. IMMUNOTHERAPY

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Efficient Elimination of Cells from Patients with Different AML Subtypes by Dual-Targeting Triplebody 33-16-123

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Introduction: A clinical candidate for triplebody 33-16-123 (SPM-2) has been identified and a process for its production has been developed. This protein, designed for the treatment of AML, carries antibody-derived binding sites for CD33 and CD123 and can bind one copy each of these antigens on the same target cell. This mode of "dual targeting" focusses the attack on antigen double-positive AML blasts and on AML leukemia stem- and progenitor cells, because they carry an elevated surface antigen density of CD123. The protein also carries a binding site for CD16, the Fcγ RIII receptor and recruits NK cells as cytolytic effectors.
Purpose: The purpose of this study was to investigate, whether NK-cells together with this agent are able to lyse blasts from patients with a broad range of different AML subtypes and whether we can find first indications for lysis of AML stem- and progenitor cells.
Results: Primary blasts from 29 patients with many different AML sub-types were lysed very efficiently with NK cells from healthy donors and in one case with autologous NK-cells from a patient obtained in a first remission.
Conclusion: The doses for half-maximum lysis in cell culture cytotoxicity assays (EC50) were in the pico- and nanomolar range, suggesting very high antileukemic efficacy for planned future clinical use.
Conflict of interest: None

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Preliminary Biomarker and Pharmacokinetic-Response Relationships in a Phase 1 Study of AMG 330, a Bispecific CD33 T-Cell Engager (BiTE®) Antibody Construct, in Patients (PTS) with Relapsed/Refractory (R/R) Acute Myeloid Leukemia (AML)

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Purpose: AMG 330 is a CD33 BiTE® under investigation in an ongoing phase 1 first-in-human (FIH) study in pts with r/r AML. We assessed the correlation between pharmacokinetic (PK) and hematologic parameters and response to AMG 330 in the FIH study.
Methods: Pts (≥18 y) with r/r AML, ≥5% blasts, and ECOG PS ≤2 enrolled sequentially to receive AMG 330 by continuous intravenous infusion at escalating doses (0.5, 1.5, 4, 10, 30, 60, 120, 240, and 480 µg/day), a run-in of 10 µg/d then the target dose, or a two-step regimen of 10 µg/d, 60 µg/d, and then the target dose. The relationship between hematologic markers in peripheral blood (PB) or AMG 330 exposure (steady-state concentration [C_{ss}]) and clinical response per IWG criteria was assessed by multivariate logistic regression.
Results: Of 40 pts who received AMG 330 in this ongoing study, 5 (12.5%) had evidence of response (CR, n=2; CRi, n=2; morphologic leukemia-free state, n=1). Response was significantly associated

with higher peak IL-10 (P=0.02), and there were trends for baseline lower tumor burden (P=0.07) and CD33+ AML cells (P=0.10) and higher E:T ratio (P=0.14) and CD8% of WBC (P=0.24). There was no correlation between response and PD-1+ on CD4+ and CD8+ T cells, magnitude of T cell activation (CD69 and CD25), or peak IL-6. PK data was available for 36 pts, of whom 17 had C_{ss} data. Responders typically had higher mean (SD) AMG 330 C_{ss} than non-responders (1.7 [1.3] vs 1.3 [1.0] ng/mL).

Conclusion: In this preliminary exploratory analysis, lower tumor burden and target expression, as well as higher E:T ratio and peak IL-10 levels in PB appeared to be associated with clinical response in pts with r/r AML.

Conflict of interest: C.E Dos Santos, A. Anderson, M. Yago, R. Lesley, M. Lutteropp and B. Mehta are employees and stock holders of Amgen

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Enhanced Phagocytic Eradication of Leukemia Cells by Combination of an FC-Engineered CD19 Antibody with CD47 Immune Checkpoint Blockade

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Introduction: Immunotherapies are on the rise in the treatment of B cell precursor acute lympho-blastic leukemia (BCP-ALL). Beside the bispecific [CD19×CD3] T cell engager blinatumomab, the CD22 antibody drug conjugate inotuzumab ozogamicin and CD19 targeting chimeric antigen receptor T cells (tisagenlecleucel) also Fc engineered CD19 antibodies with improved Fcγ receptor (FcγR) binding may have potential.

Method and results: Here, in an attempt to enhance CD19 antibody efficacy, we analyzed blockade of CD47, an ubiquitously expressed `don`t eat me!´ signal, which may compromise CD19 antibody efficacy by ligation of the myeloid inhibitory signal regulatory protein α (SIRPα). For specific blockade of CD47, which was highly expressed in BCP-ALL cells (2 - 9 × 10^4 molecules/cell), a modified CD47 antibody (CD47-IgG2σ) devoid of FcγR binding and effector functions was generated by expression in CHO cells. In addition, the Fc engineered antibody CD19-DE (modifications: S239D/I332E) as well as its native variant CD19-IgG1 were expressed. CD19 antibodies had equal avidity to CD19, but CD19-DE showed enhanced binding to FcγRIIIA, which is displayed by macrophages and natural killer (NK) cells. CD19-DE had an increased potency to trigger antibody-dependent cellular phagocytosis (ADCP) by macrophages and to induce antibody-dependent cell-mediated cytotoxicity by NK cells when employing BCP-ALL target cells. With unpolarized (M0) macrophages CD47-IgG2σ alone was not effective, but significantly enhanced ADCP of BCP-ALL cell lines when combined with CD19 antibodies. Thus, CD19-IgG1, which alone had only moderate effects, triggered considerable ADCP upon CD47 blockade. Impressively, CD47-IgG2σ even enhanced ADCP by CD19-DE, augmenting mean ADCP from ~30% to ~60% in assays with Nalm-6 cells. CD47-IgG2σ also enhanced ADCP by polarized inflammatory M1 or regulatory M2 macrophages. Finally, CD47-IgG2σ also proved efficacy when BCP-ALL patient cells were analyzed.

Conclusion: In conclusion, CD47 antibodies may represent an attractive strategy to further improve CD19 antibody therapy in BCP-ALL. In particular, combination of CD47 blockade with Fc engineered antibodies appears promising and deserves further *in vivo* testing.

Conflict of interest: None

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Anti-Human CD117 CAR T-Cells Efficiently Eliminate Hematopoietic Stem and CD117-Positive AML Cells

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Introduction: Acute Myeloid Leukemia (AML) originates from immature hematopoietic stem and progenitor cells (HSPC). While some AML are curable, disease relapse occurs in most of patients upon application of current standard chemotherapy approaches. Recently, eradication of leukemia or lymphoma cells by immunologically targeting lineage specific surface antigens (e.g. CD20, CD19, BCMA) has been achieved. To date, however, the search for AML-specific surface antigens has remained largely elusive. We thus propose in a proof of concept to target the HSPC antigen c-Kit (CD117) expressed by healthy HSPC as wells as by leukemic blasts in >90% of AML patients with CD117 specific CAR T cells,

terminate the response, and subsequently conduct healthy/allogeneic HSC transplantation.

Methods: We generated a lentiviral vector which incorporates the anti-CD117 CAR followed by a T2A ribosomal skip sequence and RQR8 as selection marker and depletion gene. Human CD117 was cloned in human CD117 negative HL-60 AML cells and cell lines with stable expression of CD117 at various levels were derived from these.

Results: T-cells were isolated from healthy donors or AML patients in complete remission, respectively, and exhibited sustained growth after activation with recombinant human IL-2 and CD3/CD28 beads. In vitro, CAR T-cells eliminated more than 90% of $CD117^{high}$ leukemia cell lines within 24 hours at effector-to target ratios (E:T) of 4:1 and 1:1 and more than 50% at E:T of 1:4. CAR-mediated cytotoxicity correlated with levels of CD117 surface expression as the elimination of $CD117^{low}$ target cells was less efficient compared to $CD117^{high}$ and $CD117^{intermediate}$ cells. With primary cells, anti-CD117 CAR T-cells effectively depleted >90% of $lin^{-}CD117^{+}CD34^{+}CD38^{+}$ and >70% of $lin^{-}CD117^{+}CD34^{+}CD38^{-}$ cells from healthy bone marrow in vitro. Similarly, patient derived leukemic blasts were eliminated by autologous anti-CD117 CAR T-cells. To determine effectivity of anti-human CD117 CAR T-cells in vivo, humanized mice were engrafted with umbilical cord blood $CD34^{+}$ cells. A single injection of $2x10^{6}$ anti-CD117 CAR T-cells resulted in >90% depletion of $CD117^{+}$ cells in the bone marrow within 6 days. Finally, humanized mice transplanted with primary $CD117^{+}$ AML were treated with patient-derived autologous CAR T-cells. At 6 weeks after injection of CAR T-cells, >98% of hu-CD45 $CD117^{+}$ cells were depleted.

Conclusions: We provide proof of concept for the generation of highly-potent CAR T-cells re-directed against CD117 from healthy human donors and AML patients. Strategies for the complete elimination of CAR T-cells are required before translation of this approach into a clinical setting for HSPC-disease therapy or HSCT conditioning.

Conflict of interest: None

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ADGRE2 Shows a Favorable Expression Profile for CAR-Targeting in Acute Myeloid Leukemia

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Background: ADGRE2, CCR1, CD70, and LILRB2 have been recently suggested as candidate chimeric antigen receptor (CAR) targets for engineered T cells in acute myeloid leukemia (AML) patients.

Aim: To validate the expression pattern of the recently identified candidate CAR targets ADGRE2, CCR1, CD70, and LILRB2 on leukemic blasts in a cohort of newly diagnosed AML patients.

Patients and methods: 150 patients with de novo (n=124) or secondary (n=26) AML were included in the analysis. Molecular analyses were performed by next-generation sequencing (NGS) with a panel of 46 genes. Bone marrow or peripheral blood samples from the time of first diagnosis were obtained to perform multi-color flow cytometry analysis evaluating the expression levels of ADGRE2, CCR1, CD70 and LILRB2 antigens on the surface of myeloid blasts. Patients with expression of the marker on ≥20% of blast cells were defined positive.

Results: ADGRE2, CCR1, CD70 and LILRB2 were detected on myeloid blasts in 99%, 69%, 21%, and 23% of patients with a median expression of 91% (range 16-99%), 33% (range 0-86%), 9% (range 0-55%), and 8% (range 0-90%), respectively. ADGRE2, CCR1, CD70 and LILRB2 were expressed on CD3+ T cells in 19% (range 0-64%), 12% (range 0-73%), 5% (range 0-83%), and 1% (range 0-18%) of patients with positive marker expression, respectively. The proportion of patients with ≥80% expression on blasts was 69%, 1%, 0% and 3% for ADGRE2, CCR1, CD70 and LILRB2, respectively. These patients showed an ADGRE2, CCR1 or LILRB2 expression on CD3+ T cells of <20% in 42%, 0%, and 100% and of <1% in 1%, 0%, and 25%, respectively. There were no significant differences in the distribution of age, type of AML, sex, cytogenetic risk according to 2017 ELN classification or blood cell count between CCR1, CD70, and LILRB2 positive compared to negative patients. In CEBPA mutated patients the mean expression of CCR1 and CD70 was higher compared to wildtype patients (43% vs. 31%, P=0.001 and 21% vs. 12%, P=0.031, respectively). However, no other mutations correlated with the expression of the candidate CAR targets. Overall survival, event-free and relapse-free survival were also similar for patients with and without expression of CCR1, CD70 and LILRB2.

Conclusions: The protein expression profile of candidate CAR targets ADGRE2, CCR1, CD70 and LILRB2 on AML blasts was less favorable than previously reported in our large cohort of primary AML samples. Expression levels were

mostly not associated with patient characteristics or outcome. Our findings favor ADGRE2 as a potential CAR target as it had the highest ratio of a high expression on myeloid blasts and a low expression on T cells in a significant number of patients.
Conflict of interest: none

VI. PEDIATRIC ACUTE LEUKEMIAS

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NG2 Predictive Value for Presence of *KMT2A*-Rearrangements Differs and Depends on Age and Acute Leukemia Type

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Introduction: *KMT2A* gene rearrangements (*KMT2A*-r) in acute leukemia (AL) can occur with more than 90 partner genes. Therefore, surrogate screening for *KMT2A*-related markers could be useful to distinguish a group of patients that are highly likely to have *KMT2A*-r. Determination of these patients is necessary for further thorough molecular analysis. NG2 expression is considered to be the most specific immunophenotypic feature of 11q23-associated AL. Although association of NG2 expression with *KMT2A*-r is well known for AL in infancy, similar data for older children is controversial.
Purpose: The purpose of the study was to evaluate a predictive value of NG2 expression for presence of *KMT2A*-r in various groups of children with AL.
Patients and methods: 269 children under 18 years with NG2 expression by at least 1% of leukemic population were studied. Acute lymphoblastic leukemia (ALL) was diagnosed in 162 cases while 107 patients had acute myeloid leukemia (AML). In 85 children with ALL (52.5%) and 71 (66.4%) with AML *KMT2A*-r were detected. Using ROC-analysis, we defined percentage of NG2-positive cells with the best ability for *KMT2A*-r(+) and *KMT2A*-r(-) groups discrimination. Predictive capacities of defined threshold levels and two standard cut-offs (20% and 10%) were compared. Association of NG2 expression level with presence of *KMT2A*-r was studied using MESF values.
Results: According to age (below 1 year vs 1-18 years) and diagnosis (ALL vs AML) patients were divided into four groups. In infants ROC-analysis showed that 5% of NG2-expressing cells (1.00 specificity and 0.96 sensitivity) for ALL (n=53) and 10% (1.00 specificity and 0.84 sensitivity) for AML (n=23) displayed the best ability for prediction of 11q23 aberrations. In older children, the correlation was less obvious, although NG2 still could be used as screening marker to narrow the group of patients with suspect for *KMT2A*-r. 11% NG2 level in ALL (n=109, 0,853 specificity and 0,747 sensitivity) and 13% of NG2-positive cells in AML (n=84, 0,750 specificity and 0,758 sensitivity) were defined as the best cut-off points. Thus, we suggest applying 10% threshold for ALL and AML children older than 1 year as a screening tool for prediction of *KMT2A*-r. MESF cut-off values for all four groups were close to 1000, but the defined sensitivity was lower comparing to NG2-positive cells percentages.
Conclusion: NG2 predictive value differs and depends on age and AL type. Our data indicates usefulness of NG2 evaluation for subsequent precise identification of children with *KMT2A*-r in all patients groups, that is crucial for correct risk groups stratification.
Conflict of interest: None

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The *MLL*-Recombinome of Pediatric Acute Leukemia in the Russian Federation

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Purpose: Histone-lysine N-methyltransferase 2A gene rearrangements (*KMT2A*-r) are common genetic events in pediatric acute leukemia (AL). They demonstrate the highest incidence in infant AL thus creating a distinct and well-described subset of *KMT2A*-associated ALs with *KMT2A-AFF1* fusion gene prevalence. However, *KMT2A*-r incidence drops down with age, and a great variety of different translocation partner genes (TPs) emerges. So, in the recent study we aimed to characterize *KMT2A*-r pattern in pediatric AL patients over 1 y.o. in Russian Federation.
Results: The predominant diagnosis was acute myeloid leukemia (AML; 154 cases, 61.4%) followed by acute lymphoblastic leukemia (ALL; 89 cases, 35.5%). The most common AL variants in each group were acute monocytic leukemia (64 cases, 55.2% of AML) and pro-B ALL (29 cases, 56.9% of ALL). The maximum *KMT2A*-r

incidence fell within 1-2 y.o. in both in AML and ALL with additional peaks at 9-15 y.o. in AML.
Top widespread aberrations included t(9;11)(p21;q23)/*KMT2A-MLLT3* (96 cases, 38.2%), t(4;11)(q21;q23)/*KMT2A-AFF1* (34 cases, 13.5%), t(10;11)(p12;q23)/*KMT2A-MLL10* (34 cases, 13.5%), t(11;19)(q23;p13.3)/*KMT2A-MLLT1* (28 cases, 11.2%). We characterized several rare *KMT2A*-r as well: t(X;11)(q24;q23)/*KMT2A-SEPT6* (4 cases), t(11;17)(q23;q12-21)/*KMT2A-MLLT6* (2 cases), t(10;11)(p12;q23)/*KMT2A-NEBL* (2 cases), t(5;11)(q31;q23)/*KMT2A-ARHGAP26* (1 case), t(11;22)(q23;q11)/*KMT2A-SEPT5* (1 case), t(11;16)(q23;q23) / *KMT2A-USP10* (1 case). The rare *KMT2A*-r were seen exclusively in AML.
We also analyzed rearranged *KMT2A* breakpoint location in 90 cases. It resided predominantly within introns 9 (38 cases, 42.2%) and intron 10 (36 cases, 40.0%) as numbered by Meyer et al. [Meyer et al., 2006]. Intron 11 breakpoint location which was shown to be associated with poor prognosis in children under 2 y.o. [Emerenciano et al., 2013] was only found in 9 cases (10.0%) in our cohort suggesting its association with younger age.
Conclusions: *KMT2A*-r in pediatric patients over 1 y.o. in Russian Federation demonstrate higher incidence in AML with *KMT2A-MLLT3* fusion gene prevalence. The work was supported by RFBR grant №17-29-06052.
Conflict of interest: None

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INTRON Retention in KMT2A-MLLT3 Fusion Transcript in Pediatric Acute Leukemia

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Purpose: *KMT2A* gene rearrangements are frequently found in pediatric acute leukemia. They are thought to be primary leukemogenic events and serve as excellent leukemic clone markers both for initial diagnosis and MRD monitoring. However, in some cases common methods of *KMT2A* rearrangement analysis give discordant results due to modified fusion transcript structure.
Results: T(9;11)(p11;q23) was found by G-banded karyotyping and FISH. At the same time conventional real-time RT-PCR showed negative results. Further analysis revealed KMT2A-MLLT3 fusion transcript showcasing *KMT2A* intron retention.

Karyotype	46,XX,del(7p)?,t(9;11)(p21;q23)[5]
FISH Kreatek	nuc ish 9p21 (MLLT3 x 3), 11q23 (MLL x 3) (MLL con MLLT3 x 2) [90/100]
KMT2A-MLLT3 transcript	KMT2A exon 9 + KMT2A intron 9 (part) + MLLT3 exon 7 (part) + MLLT3 exon 8

This transcript demonstrated inclusion of *KMT2A* intron 9 part and loss of *MLLT3* exon 7 part, nevertheless, they were fused in-frame. Longer transcript was tricky to uncover with conventional real-time RT-PCR without additional elongation. The inserted intron sequence comprised an AluSx1 repetitive element fragment. This suggested that the aberrant transcript was formed due to exonization of ancestral intronic sequence, a mechanism referred to as Class A intron retention event. On the other hand, *KMT2A* reciprocal translocations commonly arise from non-homologous recombination driven by intronic Alu repeats. The detected splice site substitutions hint that both translocation and Alu-derived chimeric exon took place in the current case. Intron retention is a common way of gene transcription regulation both in physiological state and in disease, but its role in the fusion transcripts' biology is to be further studied.
Conclusions: Here we for the first time show the intron retention phenomenon in KMT2A-MLLT3 fusion transcript. Intron retention rather than rare fusion partners must be considered when cytogenetical and molecular diagnostics show discordant results in pediatric acute leukemia with *KMT2A-MLLT3* fusion gene. The work was supported by RFBR grant №17-29-06052.
Conflict of interest: None

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Verification of Pediatric *BCR-ABL1*-LIKE ALL Cases by Real-Time PCR

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Aims: To identify *BCR-ABL1*-like pediatric ALL patients by real-time PCR.
Patients and methods: The study was done on initial BM samples of 144 primary pediatric BCP-ALL patients. Positive cohort included 10 *BCR-ABL1*-positive ALL patients, negative cohort

consisted of 54 cases with known structural or numerical aberrations and 6 Down syndrome (DS-ALL) patients. The rest 73 cases were called 'B-others' ALL and designated as training cohort. Based on previously published data we assessed expression *IGJ, SPATS2L, MUC4, CRLF2, CA6* by real-time PCR. *IKZF1* status was evaluated by MLPA. Presence of *ABL1, ABL2, CRLF2, IgH, JAK2, PDGFRb/CSFR1* gene rearrangements were estimated by FISH. Prognostic value of *BCR-ABL*-like profile was estimated in 66 'B-others' patients uniformly treated according to the ALL-MB 2008 protocol.

Results: Hierarchical cluster analysis and PCA showed that 16 examined samples were clustered together with 9 *BCR-ABL1*-positive ones. Among them there were 3 DS-ALL patients, 1 iAMP21, 1 t(12;21),11 patients from 'B-other' ALL group. *IKZF1* deletions and TK fusion genes were more frequent in *BCR-ABL1*-like group in comparison to non-*BCR-ABL*-like B-others (53% vs 12%, and 63% vs 0%, correspondingly; $p<0.001$ in both cases). *BCR-ABL1*-like profile was associated with female gender ($p=0.003$), initial WBC $\geq 30*10^9$ /L ($p=0.013$). EFS of *BCR-ABL1*-like patients was lower in comparison to non-*BCR-ABL1*-like 'B-other' patients enrolled into ALL-MB 2008 protocol (0.28±0.17 vs. 0.93±0.04, $p<0.0001$) while cumulative incidence of relapse was significantly higher (0.57±0.19 vs. 0.02±0.02, $p<0.0001$). The worst outcome was noted in case of combination of *BCR-ABL1*-like profile and *IKZF1* deletions (EFS 0.00), while patients with isolated *BCR-ABL1*-like profile doing much better (EFS 0.67±0.22). Poor EOI flow-MRD response (>0.1%) together with *BCR-ABL1*-like profile identified a group of patients with dismal outcome (EFS 0.00). Overall accuracy of *BCR-ABL1*-like profile for relapse prediction was 0.892.

Conclusion: Thus, we showed that real-time PCR technology based on expression data of 5 genes allowed detecting the *BCR-ABL1*-like ALL patients with similar clinical characteristics, genetic parameters and treatment outcome to ones revealed by microarray, NGS or TLDA techniques. We have introduced detection of *BCR-ABL1*-like profile by PCR as first screening step in the identification of this cohort of patients within Moscow-Berlin study group BCP-ALL patients.

Conflict of interest: None

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Efficacy of Epigenetic Therapy with Intensive Chemotherapy in the Treatment of Childhood Acute Myeloid Leukemias

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Introduction: The results of treatment children with acute myeloid leukemia (AML) are not satisfied yet. The standard chemotherapy allows achieving complete remission in 92-96% of patients, but disease free survival (DFS) and event free survival (EFS) are not good yet.

Aim: In a new study – AML NII DOG 2012 – the specific aim was to explore effectiveness and toxicity demetilating medicine (Decitabine) and inhibitors of histonedeacetilase (HDAC) to find the place in standard chemotherapy.

Patients and methods: From 01.2013 to 03.2018 23 patients (grou-1) were enrolled into NII DOG AML 2012 study. Chemotherapy consisted on 5 courses for HR and IR (AIE, HAM, AI, hAM, HAE) and 4 courses for SR (AIE, HAM, hAM, HAE). Epigenetic therapy consisted of Valproic acid (VA) days1-78, All Trans Retinoic Acid (ATRA) 1-43 days and from the day one to day 14 of the every course chemotherapy and Decitobine on day 16 after beginning of induction 20 mg/m^2 for 5 days. At the same time 52 pts were treated by the same chemo without epigenetic therapy (group-2). There were no any significant differences in age, sex and risk groups between two cohorts of pts.

Results: Complete remission was achieved in 100% of group-1 comparing to 92,9% of group-2 ($p=0,04$). Three pts achieved CR after getting Decitobine without chemotherapy in timing regime. Moreover, 29 pts from group-2 achieved CR after timing induction therapy (AIE plus HAM). Median duration of neutropenia was 34,4±1,8 days, and group-2 – 30,0±1,0 ($p=0,14$). Five years EFS of group-1 was 66,7±12,4% with median follow up 42,5±4,4 mo, and group-2 – 54,2±8% n=52, median follow up 41,73±4,3 mo. OS was 81,2±9,8% median follow up 46,7±3,9 mo and 71,4±6,6% n=52, median follow up 54,4±4,2 mo. None of the pats from group-1 got HSCT but 6 pts (12%) were undergone by HSCT in group-2.

Conclusion. Thus, epigenetic therapy increased rate of CR, EFS and OS in children with AML, and did not increase toxicity. Decitobine allowed achieving CR even in patients with poor response on day +15 and avoided timing chemotherapy. Demetilating medicine has to be used during aplasia and HDAC inhibitors – during the chemotherapy program.

Conflict of interest: Supported by The American Woman Organization and "Nastenka" Charitable Foundation Helping kids with Cancer

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Analyzing Transcriptional Profiles of Childhood ALL at Single Cell Resolution

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Introduction: Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, and despite high cure rates, relapse occurs in up to 20% of all cases and is associated with poor prognosis. Early identification of relapsing cases, especially when lacking high risk features, is therefore of utmost importance. For this purpose, we used single cell RNA sequencing (scRNA-seq), which has been established as a powerful genome-wide method to resolve the heterogeneity of cells within a given tissue. Intra-tumor heterogeneity is linked to therapy resistance and the ensuing development of relapse.
Aim and method: We previously performed scRNA-seq of ALL patient *cells* to characterize therapy resistant cells (Ebinger et al., 2016) and compare them to slowly proliferating cells found in a PDX treatment model. Here we performed scRNA-seq of B cell precursor (BCP)-ALL cells at diagnosis for 12 pediatric patients (6 relapsing and 6 non-relapsing cases) using the mcSCRB-seq protocol (Bagnoli et al., 2018) to get highly sensitive measurements of single cell mRNA levels.
Results: A first comparison of relapsing and non-relapsing ALL cases revealed differences in mappability of the generated sequencing reads and differences in transcriptome profiles. To confirm these findings, diagnosis samples of an additional cohort of 12 ALL patients will be analysed, leading in total to over 3400 scRNA-seq profiles of 24 different ALL patients.
Conclusion: With this approach, we expect to gain a deeper understanding of differences between resistance and sensitivity of ALL cells to chemotherapy. Ultimately this knowledge might be utilized to inform novel treatment modalities.
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Conflict of interest: none

The Definition of the MRD is the Main Stratification Tool for Risk Groups Detection in Children with B-ALL

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Introduction: Despite at the present 98% patients with acute lymphoblastic leukemia (ALL) achieve complete clinical remission, significant proportion of them ultimately relapse. One of the most important causes of relapse is persistence of residual malignant cells that are undetectable by morphological study of bone marrow (BM) aspirate. Several studies have shown that detection of minimal residual disease is an independent risk parameter of high clinical appropriateness.
Aim: To determine minimal residual disease in children with B-precursor ALL (B-ALL), to use this for change risk group stratification.
Patients and method: From 2010 to 2018 115 pediatric patients with B –ALL were treated by protocol ALL IC BFM 2009. Median age was 5,2 years (from 1 to 16). Male – 51, female – 64. The diagnosis was made by standard morphological analysis, and by flow cytometry immunophenotyping and cytogenetic study. 107 patients had "common"-ALL, 2 – pre-B ALL and 6 – pro-B ALL. According to the protocol risk stratification was based on initially characteristics of patient (age, WBC rate, genetic translocations and prednisone response). Initially forty patients had standard risk group (SR), 70 – intermediate risk (IR), 5 – high risk (HR). MRD by flow was detected at day 15 of induction therapy.
Results: Less than 5% of blast cells in BM (M-1 morphology response) on the day 15 was in 89 (77,4%), M-2 (5–25% of blasts) – in 20 (17,4%) patients and M-3 (>25%blasts) – in 5(4,3%) patients. MRD level <0.1 % was detected in 54 (47%) patients, MRD >0,1 –<10% was in 47(40,9%) patients and MRD ≥10% – in 12 (10,4%). In accordance with MRD results, risk group was changed in 21 patients: 1 patient was moved from standard to high risk, 12 patients – from SR to IR group, 8 patients – from IR to HR. All 21 patients achieved complete remission and are still alive without relapse more than 5 years. So, after restratification 5y-EFS was 100% (n=25) in SR, 81.7±6,3% (n=68) in IR, and 69.8±13,7% (n=21) in HR. Death in induction amounted to 0,86% (n=1)
Conclusion: These results confirm the importance of MRD analysis for risk adapted treatment childhood B - ALL to use it to determine the intensity of postinduction therapy.
Conflict of interest: none

Epigenetic Therapy Allows to Improve the Survival of Children with Acute Myeloid Leukemia

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Introduction: Epigenetic changes play an important role in the development of acute myeloid leukemia (AML). Epigenetic changes are potentially reversible, which makes them attractive for therapeutic intervention. DNA methylation inhibitors and HDAC inhibitors are synergistic in activating gene expression. A combination of epigenetic drugs with chemotherapy should show activity in AML.

Patients and method: Forty one children with newly-diagnosed AML were included into AML-2012 protocol. There were 21 males and 20 females. The average was 6.6±1.3 years (variation range 6 month to 16 years). Risk-group stratification was based on cytogenetic abnormalities present in the leukemic blasts in combination with early response to treatment and complete remission after two courses chemo. There were Standard, Intermediate, and High risk groups in clinical trials.

AML-2012 protocol included one course of the induction for Standard and two for Intermediate and High risk groups. Patients got two courses the intensification and one consolidation chemo after induction therapy.We used high doses of cytarabine and anthracycline antibiotic for intensive chemo. Then the patients got the maintenance therapy during one year. We added Valproic acid (VPA), All-trans-retinoic acid (ATRA), Decitabine or Azacitidinum to chemo. VPA was given at a dose of 25mg/kg per os over the whole period of treatment. Patients received Decitabine 20mg/m^2 intravenously over one hour days -5 to -1 before (group 1) or +16 to +20 (group 2) days in the first induction course or Azacitidinum 75mg/m^2 +16 to +20 days (group 3). ATRA was given 45mg/m^2 per os for 14 days each courses of chemo and each month maintenance therapy.

Results: We achieved complete remission in 92,1% patients, all children who got Decitabine days +16 to +20 (group 2) had remission. Probability of 3-year disease free survival following AML-2012 was 50.9±11.7% (median 36.6±2.4 month), for group 1 – 40.0±21.7%, group 2 – 66.0±10.1% and group 3 – 20.5±11.7%.

Conclusion: Epigenetic therapy combined with chemo allowed to improve survival of patients with acute myeloid leukemia.

Conflict of interest: None

MIR-497~195 Cluster Has Tumor Suppressive Function in Pediatric ALL

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Background: Despite recent advances in the treatment of B-cell precursor acute lymphoblastic leukemia (BCP-ALL), relapse is associated with poor prognosis, indicating the need to better understand the underlying biology. We previously showed that rapid engraftment of ALL cells transplanted onto NOD/SCID mice is associated with poor patient outcome.

Aim and method: Here, we analyzed the expression and role of microRNAs (miRNAs) in BCP-ALL. We performed small RNA sequencing of 13 BCP-ALL patient-derived xenograft samples and identified 13 miRNAs differentially expressed between samples with different engraftment phenotypes.

Results: The tumor suppressor miR-497-195 cluster was downregulated in samples with rapid engraftment/early relapse. As possible regulation mechanism, we found an association of methylation of the respective promoter and miR-497~195 expression. Further, miR-497 showed lower expression in samples derived from patients suffering from early relapse as compared to late or no relapse in an extended xenograft cohort, indicating an association of miR-497 high expression and more favorable outcome. We investigated the impact of miR-497~195 on *in vivo* growth of ALL cells by lentiviral overexpression and transplantation of transduced cells into NOD/SCID mice. In cell line EU-3 miR-497~195 overexpression led to a reduction of tumor load in the spleen of transplanted mice compared with control. We overexpressed the cluster in a BCP-ALL xenograft sample with low expression of miR-497~195 and rapid engraftment. Mice transplanted with miR-497~195 overexpressing cells had reduced tumor load of transduced cells in spleen and bone marrow as compared to control. When the cells isolated from these mice were transplanted onto new recipients, miR-497~195 overexpression caused delayed leukemia development and prolonged survival. Finally, gene expression profiling by microarrays was performed on transduced blasts isolated from transplanted mice. Genes downregulated upon miR-497~195 overexpression were enriched for Reactome pathways related with cell cycle progression. In line, first functional data showed decreased cell viability and proliferation upon miR-497~195 overexpression.

Conclusion: Altogether, our data show association of high expression of miR-497~195 with a better

outcome in BCP-ALL, and promoter methylation as a possible repression mechanism. Overexpression of miR-497~195 reduced tumor growth and prolonged survival *in vivo*, inhibiting cell cycle progression and indicating a tumor suppressor role for miR-497~195 in BCP-ALL.
Conflict of interest: none

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***TP53* Alterations and Gene-Expression Profiling of P53 Pathway Genes in Pediatric Acute Myeloid Leukemia**

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Background: *TP53* mutations occur in 5-8% of adult acute myeloid leukemia (AML) patients and confer dismal outcome. Furthermore, studies have shown that p53 pathway dysfunction is highly prevalent in AML, independent of *TP53* mutations.
Aim: This study aims to gain more insight on the prevalence of *TP53* mutations and clinical implications of aberrant p53 pathway gene expression in pediatric AML.
Materials and methods: In 228 pediatric AML patients, sequencing of exons 5-9 of TP53 was performed, covering all mutational hotspots for AML. Clinical data and other recurrent molecular aberrations were available for these patients. Furthermore, gene expression profiling was performed.
Results: Two patients (1%) had a heterozygous missense exon mutation and four patients (2%) had a 17p deletion of the *TP53* gene. One of these mutations was in a hotspot location in exon 8 (codon 282). However, this specific amino acid change was not previously described in AML (arginine to glutamine). Patients with *TP53* alterations had significantly more often adverse cytogenetic abnormalities (66% vs. 19%, P=0.013) and complex karyotype (50% vs. 8%, P=0.002) compared to $TP53^{wild\text{-}type}$ patients. Analysis of gene expression profiles showed a specific cluster of patient samples with overexpression of *MDM2*, which was associated with inferior overall survival. *MDM4* and *TP53* overexpression did not have adverse clinical implications in this cohort. We did not observe any correlation between *MDM2* and *TP53* expression.
Discussion: Mutations in *TP53* are rare in pediatric AML, supporting the suggestion that *TP53* mutations are dependent on environmental carcinogens and aging. Even though in this study few patients had *TP53* mutations, significantly more cytogenetic abnormalities involving the *TP53* gene were found, suggesting that the biology and prognostic significance of *TP53* alterations is similar to adult AML. Moreover, in our data, overexpression of the negative regulator of *TP53, MDM2,* is an independent prognostic factor. This is indicative that p53 pathway dysfunction can be of great importance independent of *TP53* status.
Conclusion: These results need to be validated in larger cohorts and may lead to improved risk stratification and related potential therapeutic strategies.
Conflict of interest: none

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Hyperleukocytosis in Children with Acute Myeloid Leukemia: A Single-Center Experience

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Introduction: the prognostic role of hyperleukocytosis (HL) in acute myeloid leukaemia (AML) in children is a matter of a discussion
Patients: from 2012 to 2017, 185 patients (pts) were treated for AML in our center. Hyperleukocytosis was defined accordingly to AML-MM-2006 protocol: leukocyte level (Le) > 50 x 10^9/l in pts with M4 and M5 FAB variants and >100 x 10^9/l for all other variants. Thirty six out of 185 pts had HL (19,5%). Median age was 7y 8m (7d – 18y). Median Le was 97 x 10^9/l (50 – 428 x 10^9/l).
Results: according to the stratification, 4 pts (11%) with HL were considered at standard risk, 8 (22%) - at intermediate risk and 24 (67%) - at high risk. The most common variant was M5 – 18 (50%) pts. There were 14 (39%) pts with M4, 1 (3%) pts with M2 and 3 (8%) – with M1. Considering clinical features, 31 pts had hepatomegaly and 24 had splenomegaly ($p = 0,025$ and $p = 0,006$ respectively). Extramedullary lesions were observed in 9 (25%) patients. Eleven (30%) pts had neuroleikemia ($p = 0,8$). The most common cytogenetic abnormality was *MLL* rearrangements, which were observed in 15 (42%) patients. Thirty-five patients with HL received cytoreduction course: daunorubicin 22,5 mg/m^2/d and etoposide 50 mg/m^2/d, both intravenously with continuous infusion until Le was < 50 x 10^9/l. After that, all patients received ADE part of induction. The second part of induction (HAM course on the day 14 from the beginning of ADE) was skipped in 15 patients (death before the day 14 – 3 patients, *inv16* – 3 pts, life-threatening

infections – 7 pts, brain haemorrhage – 1 patient, severe cardiac insufficiency – 1 patient). Remission was achieved in 27 (75%) out of 36 pts. Nine patients didn't achieve remission after the induction course: 3 died and 6 had refractory disease. Nine (33%) out of 27 patients in I CR relapsed. HSCT was performed in 23 pts: MRD in 3 patients, MUD in 9 pts and haplo in 11 pts. Thirteen (36%) out of 36 patients with HL died: 4 (30%) – due to leukostasis complications (3 – before remission, 1 – in I CR), 6 (43%) – due to AML progression, 3 (24%) – due to HSCT complications. To date, 19 patients are alive in I CR, 4 – in II CR, 13 – died. OS for HL group was 0.56 ± 0.09, for non-HL group was 0.75 ± 0.04, $p = 0,005$; EFS (HL) = 0,42 ± 0,09, EFS (non-HL) = 0,49 ± 0,04, $p = 0,026$. Also, differences in I CR achievement, median of remission length and death before remission between two groups were statistically significant ($p = 0,036$, $p = 0,028$, $p = 0,021$ respectively).

Conclusion: OS and EFS in patients with M4 and M5 were better than in patients with "true" HL (>100 x 10^9/l), OS = 0,71 ± 0,1 vs OS = 0,43 ± 0,1, ($p = 0,012$); EFS = 0,54 ± 0,1 vs EFS = 0,29 ± 0,1 ($p = 0,038$) respectively. HL significantly worsens OS and EFS in children with AML.

Conflict of interest: none

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CD1a and T-CELL Receptors Expression Predicts Outcome in Childhood T-Lineage ALL

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Introduction: Despite significant improvement of childhood acute lymphoblastic leukemia (ALL) treatment results, T-lineage ALL (T-ALL) management is still a challenge. Additional prognostic factors in this ALL type are required. Surface antigens expression profile could be one of possible risk factors in T-ALL.

Aim: Aim of the study was to investigate the prognostic significance of tumor blasts immunophenotype in childhood T-ALL.

Methods: 110 consecutive children with T-ALL (71 boys and 31 girls with median age of 8 years) were prospectively studied in two reference flow cytometric laboratories of "Moscow-Berlin" ALL MB-2015 trial. According to the protocol stratification system patients were distributed into T-low risk (T-LR, n=70), T-intermediate risk (T-ImR, n=29) and T-high risk (n=11) groups. Bone marrow blasts immunophenotype was studied by 8-10-color flow cytometry. Event-free survival (EFS) with standard error (SE) was used as the outcome parameter.

Results: Among all studied single markers only CD1a and T-cell receptors (TCR) expression was found to influence the treatment outcome. 78 CD1a-positive patients had significantly better EFS (0.83 SE 0.06) compared to 32 CD1a-negative ones (0.52 SE 0.12, p=0.043). At the same time 86 TCR-negative cases had EFS of 0.79 (SE 0.07) which was better than in 24 TCR-positive patients (EFS 0.44 SE 0.14, p=0.008). In 16 cases CD1a and TCR were coexpressed. Outcome of these patients was worse as opposed to 62 CD1a(+)TCR(-)-ones: EFS 0.57 SE 0.18 and EFS 0.92 SE 0.04 respectively, p=0.045. Similarly in CD1a-negative group TCR expression was associated with poor prognosis. 24 CD1a(-)TCR(-) children had EFS 0.62 SE 0.13 while in 8 CD1a(-)TCR(+) cases EFS was 0.21 SE 0.18, p=0.030. Thus taken together CD1a and TCR expression allowed distinguishing of three groups: favorable (CD1a(+)TCR(-), n=62, EFS 0.92 SE 0.04), intermediate (CD1a(+)TCR(+) and CD1a(-)TCR(-), n=40, EFS 0.60 SE 0.11) and unfavorable (CD1a(-)TCR(+), n=8, EFS 0.21 SE 0.18, p=0.003). This definition of patients with different outcome retained its' significance also in T-LR and T-ImR groups analyzed separately (p=0.033 and p=0.047 respectively).

Conclusion: Results of present studies indicate the significant prognostic role of CD1a and TCR expression in childhood T-ALL. Flow cytometric data could be successfully applied in patients' stratification together with conventional risk criteria.

Conflict of interest: none

MAIN SESSIONS

Main Session VII

The AML-CG Trials in APL

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Background

Despite impressive improvement of the prognosis of APL in the last decades, there are still challenges to further optimize the outcome.

First of all, the risk of early death (ED) is still high.[1] The relapse rate of high risk APL should be improved further and the optimal treatment for elderly patients is discussed. At present, a smaller number of APL patients have still an unexpectedly poor outcome, which requires a better molecular characterization of high risk disease.[2]

From 1994 to 2013, 320 patients (age 18 to 87 years) with newly diagnosed APL were registered by the German AMLCG. Of these, 271 (85%) patients qualified for inclusion in the APL-1994 and APL-2005 studies and 49 (15%) were non-eligible. In the 1994 study, the patients were treated uniformly with an intensified double induction therapy (TAD-HAM) including high dose cytosine arabinoside (ara-C), with reduction of the treatment intensity in older patients.[3,4] In the 2005-study, the AMLCG and the PETHEMA concepts were randomly compared.[5] Since 2014, 49 patients were included in the subsequent APL registry of the AMLCG and received variable therapy including ATO-based treatment.

Here, some results of the AMLCG are presented with regard to the above mentioned problems in the management of APL.

Results

Among all 196 patients (age 16 to 70 years), who received treatment according to the original AMLCG regimen (double induction with TAD-HAM, TAD consolidation, cyclic maintenance therapy), there were only 4.6% (n=9) relapses in the total group, in standard risk 3.9% (6 of 151 patients) and in high risk APL 6.6% (3 of 45 patients), respectively. The long-term overall survival (OS) in the 1994-study (n=158) was 69% at 16 years and in the 2005-study (n=38) 75% at 9 years, respectively.

Remarkably, in the age group over 60 years (n=91), 31% of patients had high risk APL and 25% were non-eligible for study inclusion. The OS at 7 years of all eligible patients over 60 years treated with the AMLCG regimen (n=56) was 45%. Elderly patients benefited from double induction, if they qualified for the second induction cycle (age adapted HAM) (n=14). In these patients, the relapse free survival at 7 years (RFS) was 83% and the cumulative incidence of relapse (CIR) 0%. After application of only one induction course (TAD) (n=32), RFS was 35% and CIR 34%, respectively).

Concerning risk of ED, we observed 11.4% ED (31 of 271 patients) in the total group, corresponding to 7.4% in standard risk and 23.1% in high risk APL, respectively. There was a negative influence of age >60 years (p=0.02) and of white blood cell counts >10,000µl. In the subgroup of study patients over 70 years with initial WBC counts >10,000µl, an extremely high ED rate of 67% was seen. Furthermore, the ED rate of the 23 patients over 60 years, who were non-eligible for study inclusion, was 48%.

Of the patients included in the ongoing AMLCG AML/APL registry, 27 patients (age 18 to 81 years) with standard risk APL were treated with ATO/ATRA according to the APL0406 protocol.[6] Of these patients, 37% were over 60 and 19% over 70 years, respectively. So far, only one patient (aged 72 years) treated with ATO/ATRA died from ED. The remaining 26 patients are in ongoing CR since two to 52 months (median 21) after achievement of CR.

As the Sanz relapse risk Score could not be reproduced in patients, who received the AMLCG regimen including high dose ara-C, a molecular score was developed on the basis of the AMLCG data as an approach for risk stratification and identification of high risk patients on the basis of molecular changes in APL.[7,8]

Conclusions

The long-term follow up of patients treated with the intensified AMLCG regimen including high dose ara-C confirms the high antileukemic efficacy of this therapy also in high risk APL. Hence, this intensified regimen might still be an option for suitable patients with ATO/ATRA resistant relapse.

ED is still a prominent risk factor, which reduces the chance of cure in APL. Older patients, in particular those with high WBC counts are mostly endangered. The preliminary results of the AMLCG registry confirm the efficacy of ATO-based therapy in all age groups of adult APL including elderly patients. Molecular risk factors might be helpful in the future to identify APL patients with poor risk.

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Conflict of interest:

MEET-THE-PROFESSOR SESSION

MEET-THE-PROFESSOR SESSION I (Gilead Sciences GmbH)

Drug interactions: Putting success of new drugs at risk

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Drug-drug–interactions (DDI) with antileukemic small molecule inhibitors (SMI) are primarily based on three different categories: (1) physico-chemical, (2) pharmacokinetic (PK), or (3) pharmacodynamic/toxicodynamic (PD/TD). As a consequence, SMI drug exposures may result in critical sub- or supratherapeutic levels in plasma over time.

Regarding physico-chemically-based DDI, negative food-effects as well as increases of gastric pH-values, e.g. by proton pump inhibitors, are of particular concern. Dasatinib-AUC has been shown to be decreased by ca. 42% during concomitant use of esomeprazole and the corresponding impairment of drug´s solubilization. Concomitant intake with Coca Cola or Betain-HCl or Ranitidine as an alternative has been proposed, when a switch within the pharmacological SMI class is not feasible.

PK-based DDI primarily involve Cyp450-mediated inhibition or induction of metabolic pathways. Particularly, if a SMI undergoes an extensive first pass effect after oral intake (e.g. Ibrutinib, Midostaurin, Bosutinib, Venetoclax or Acalabrutinib), drug-related AUC-increase may range between 5-29 x fold by concomitant use of potent Cyp3A-inhibitors (e.g. Posaconazole). Reversed signs have to be expected with concomitant potent Cyp3A-inducers (e.g. carbamazepine, rifampin, St John´s wort) with an AUC-decrease by 71-96% as a consequence.

Some SMI may themselves exert dose-dependent DDI, e.g. as CYP3A-inhibitors, when Idelalisib, Duvelisib or Ribociclib increased Midazolam AUC. If comedication undergoes an extensive Cyp3A-mediated first-pass-effects through gut and liver (e.g. lercanidpine, quetiapine, sildenafil, tacrolimus), more pronounced adverse effects might be expected.

Within the last decade, DDI-mediated impairment of transmembraner influx (e.g OATP, OCT) or efflux (e.g. ABCB1 [PgP], ABCG2) carriers have been focused as potentially important sources of treatment-related disorders, however, DDI results are primarily based on in vitro findings which makes translation into clinical practice difficult.

Concerning PD/TD-related DDI, overlapping toxicities (e.g. prolongation of QT interval) have to be kept in mind during comedication (e.g. ivosidenib and clarithromycin). Some PI3K-inhibitors (e.g. copanlisib, alpelisib) have been associated with hyperglycemia which makes special concerns in patients with diabetes necessary.

In conclusion, backgrounds and magnitudes of DDI are rather complex. As a consequence, knowledge about SMI-related biotransformation pathways and toxicity profiles appears to be mandatory for DDI prediction. In this context, currently available DDI databases, however, may not fulfill haematologists´ expectations to be broadly informed in time.

SATELLITE SYMPOSIA

SATELLITE SYMPOSIUM I
(Amgen AG)

CD33 BiTE: From Molecule to Therapy

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AMG 330 is a bispecific T cell-recruiting antibody construct directed against CD33 expressed on AML cells. CD33 (SIGLEC-3) is a lineage antigen of the myeloid compartment of haematopoiesis and expressed to a variable degree in > 90% of AML cases. At the latest through the approval of Gemtuzumab, CD33 is the best validated target antigen in AML, but like other myeloid-associated lineage antigens (e.g. CD123, CLL-1 and FLT3) is also expressed within the healthy myeloid compartment. Data on expression profiles of AML-associated target antigens including CD33 will be presented. We have developed an *ex-vivo* model for culturing primary AML cells with autologous T cells to mimic the clinical route of

application. Utilizing this system which allows AMG 330 exposure over 14 – 28 days, we were able to demonstrate high AMG 330-mediated cytotoxicity against primary AML cells. Based on this model we evaluated mode of action and mode of resistance to AMG 330. Data on the relevance of checkpoint molecules in the context of AMG 330 mediated T-cell proliferation and cytotoxicity will be presented. Meanwhile, clinical data from an interim analysis of a phase I trial I in r/r AML are available. First results of early clinical trial results on AMG 330 and other bispecific antibody constructs will be summarized.

SATELLITE SYMPOSIUM V
(Daiichi Sankyo Oncology Europe GmbH)

Targeted Therapy in AML: What do the Guidelines tell us?
Mark James Levis
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With new insights gained from next-generation sequencing (NGS) and with several new drugs recently approved, AML therapy is undergoing some dramatic changes. The term "targeted therapy" in AML is often rather loosely used to refer to any treatment not consisting of DNA-damaging chemotherapy. However, for the purposes of this presentation, we will use a more narrow definition. NGS analysis of AML specimens has uncovered an array of driver mutations. A stricter definition of a targeted therapy is one specifically designed to counter the effects of one or more of these leukemogenic drivers. The concept is not new- acute promyelocytic leukemia (APL) is defined by the presence of the PML;RARα fusion protein, and the first targeted agent in AML, all-trans retinoic acid (ATRA), specifically reversed the activity of this molecular driver and led to increased cure rates. Today, inhibitors of IDH and FLT3 mutations are recapitulating this success and confirming this therapeutic paradigm.

The initial clinical responses to these targeted agents are usually very different in nature as compared to those seen with chemotherapy. Treatment of APL with ATRA induces terminal differentiation of the malignant cells and is often accompanied by a differentiation syndrome. This phenomenon is also seen with the newer targeted agents: both IDH inhibitors and FLT3 inhibitors induce terminal myeloid differentiation within the bone marrow, as well as some version of a differentiation syndrome. Morphologically, these marrow responses can be difficult to classify, because they don't necessarily those induced by chemotherapy. However, the use of measurable (or minimal) residual disease (MRD) assays can provide important information that clarifies the depth of response and the impact on prognosis- for APL as well as for IDH- and FLT3-mutated AML.

Currently there are only single drugs approved for the treatment of IDH-mutated AML: ivosidenib to target IDH-1 mutations and enasidenib to target IDH-2 mutations. For FLT3 inhibitors, the situation is more complex. Like all tyrosine kinase inhibitors, FLT3 inhibitors are classified according to how they bind to the target. Type 1 inhibitors (midostaurin, gilteritinib, crenolanib) are generally ATP-mimetic and bind regardless of the activity state of the receptor. Type 2 inhibitors (sorafenib, quizartinib) have binding sites that extend outside of the ATP site and bind only the inactive receptor. Type 2 inhibitors are more potent, but the presence of tyrosine kinase domain (TKD) mutations (e.g., D835) abrogates much of their inhibitory activity. Type 1 inhibitors are only minimally affected by TKD mutations, but their lack of potency hampers their efficacy during periods when FLT3 ligand levels are high (such as immediately following chemotherapy). One approach would be to use a type 2 inhibitor during induction therapy, but switch to a type 1 inhibitor during a maintenance phase. While allogeneic transplant is usually the preferred consolidation for patients with FLT3-ITD mutations, the question of post-transplant maintenance (which drug, and in which patients) is an area of active investigation.
Conflict of Interest:

SATELLITE SYMPOSIUM VII
(Celgene GmbH)

How Do Genetics of AML Patients Inform Prognosis, Treatment Strategies and Follow-Up Today and Tomorrow?
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Recently, the European LeukemiaNet (ELN) recommendations for diagnosis and management of adult patients (pts) with acute myeloid leukemia (AML) have been updated. The recommendations include the revision of a number of relevant aspects, such as the spectrum of genetic testing, as well as changes in conventional care regimens. In addition, a revised risk stratification based on cytogenetic and molecular genetic aberrations was provided. Here, the ELN system was simplified by using a 3 group classification (favorable, intermediate, adverse) instead of the previous 4 group system. Based on data from recent studies molecular markers have been refined: for example, biallelic *CEBPA* mutated as well as *NPM1* mutated AML with concurrent *FLT3*-ITD low mutant/wildtype allelic ratio (< 0.5) or pts with *NPM1* mutated AML without *FLT3*-ITD are now

categorized in the favorable risk group. In contrast, AML with wildtype *NPM1* and *FLT3*-ITD high ratio (≥ 0.5) are now categorized in the adverse risk group. In addition, novel molecular markers, such as *RUNX1*, *ASXL1*, and *TP53* mutations, have been added to the adverse group, since in all studies they have been consistently associated with inferior outcome. The inclusion of these 3 markers impacts on our previous algorithms, since mutations are detectable in ~50% of the former intermediate risk I/II ELN 2010 risk groups and now stratify pts for intensive treatment such as allogeneic hematopoietic cell transplantation.

In addition, novel predictive markers have been identified which allow for the selection of pts who are eligible for molecular targeted therapies. For *FLT3*-ITD and *FLT3*-TKD mutated AML Midostaurin has been approved by FDA and EMA in 2017 for the combination treatment with intensive standard chemotherapy in first line. Recently, positive interim analysis results from the Admiral study evaluating the second-generation FLT3 inhibitor Gilteritinib versus salvage chemotherapy in 369 patients with relapsed or refractory *FLT3*-mutated AML resulted in early approval of the drug by the FDA in this indication. Similarly, the Quantum-R trial showed improved survival by Quizartinib over salvage chemotherapy in the same indication. Currently, a number of phase III studies are ongoing evaluating second-generation FLT3 inhibitors. For example, HOVON and AMLSG will launch a large international randomized first line study in *FLT3* mutated AML comparing Midostaurin versus Gilteritinib in combination with standard chemotherapy. Beside *FLT3* mutation, other predictive markers are *IDH1* and *IDH2* mutations. Here, the IDH1 inhibitor Ivosidenib as well as the IDH2 inhibitor Enasidenib were recently approved by the FDA for the treatment of refractory or relapsed AML with *IDH1* or *IDH2* mutations, respectively. First line phase III studies using Ivosidenib and Enasidenib in *IDH1* or *IDH2* mutated AML in combination with standard chemotherapy will be initiated in the first quarter 2019 by AMLSG and HOVON. CPX-351 is a liposomal formulation of daunorubicin and cytarabine that in the pivotal study showed superior survival over 3+7 induction therapy in specific high-risk subsets of AML; based on the results of this study, CPX-351 was approved by FDA and EMA. Finally, the BCL-2 inhibitor Venetoclax is a very promising drug also in AML. Venetoclax recently received approval by FDA in combination with hypomethylating agents for the treatment of older patients ineligible for intensive chemotherapy.

A prerequisite to identify pts who are eligible for such therapies is a fast molecular marker screening at the time of diagnosis. In this session the major revisions of the 2017 ELN genetic risk stratification will be discussed in the context of clinical management and treatment of AML. In addition, studies and study concepts evaluating novel molecular targeted therapies will be presented.

SATELLITE SYMPOISUM VIII
(Pfizer GmbH)

Targeting CD33 in AML
Marion Subklewe
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Immunotherapy in ALL has revolutionized treatment algorithms in ALL. In 2015, the first T-cell recruiting antibody construct, blinatomumab targeting CD19, was approved for the treatment of r/r ALL (excluding Ph positive ALL). In 2016, the first ADC, inotuzumab targeting CD22, was approved for the same indication (including Ph positive ALL). In 2017, the first CAR T cells, tisagenlecleucel targeting CD19, was approved for > 2nd line r/r ALL. The translation of the progress made in ALL to AML has been hampered by the challenge of finding suitable target antigens. CD33 is the best validated, AML-associated target antigen in AML.

Initial therapeutic approaches utilizing a conventional antibody against CD33 (Lintuzumab) to improve outcome did not show any clinical benefit. Based on the same antibody construct, an ADC was developed for the treatment of AML. Gemtuzumab ozogamicin (GO, Mylotarg) is a humanized anti-CD33 IgG4 antibody conjugated to calicheamicin. Safety concerns and failure to verify clinical benefit in a confirmatory phase III trial enrolling patients across all cytogenetic risk groups resulted in the voluntary withdrawal of GO from the market in 2010.

In recent years, both retrospective analyses and new clinical trials have been performed to unravel clinical benefits of GO in specific subgroups. A meta-analysis of five randomized controlled trials (RCTs) showed that the addition of GO to conventional chemotherapy significantly reduced the risk of relapse and resulted in an overall survival (OS) benefit mainly for cytogenetically favorable, but also for the intermediate-risk group. Accordingly, GO was re-approved for treatment of newly-diagnosed CD33+ AML in adults in combination therapy with chemotherapy (“7+3”, EMA & FDA) and as single-agent regimen (FDA only). Approval was also granted for treatment of r/r CD33+ AML in adults and pediatric patients 2 years and older (FDA only). Other ADCs directed against CD33 and alternative surface antigens like CD123 have entered clinical trials.

Emerging Treatment Strategies in AML
Carsten Müller-Tidow

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Elucidation of AML pathogenesis has paved the way for novel, more effective therapies. These approaches are urgently needed given the unsatisfactory efficacy of AML treatment in the recent past. Recently, several new drugs were approved for AML treatment.
Novel drugs acte specifically in AML subtypes with defined genetic lesions. Midostaurine and other Flt3 inhibitors improve survival in AML with FLT3 mutations. IDH inhibitors can induce extended remissions in patients with IDH mutations. Several promising approaches also exist that target a broader range of AML subtypes. These include CPX-351, a new liposomal form of Cytarabine:Daunorubicine and the anti-CD33 antibody-drug conjugate Gemtuzumab-Ozogamicin. These drugs are especially active in secondary AML and in favorable/intermediate risk AML, respectively. The BCL2 inhibitor Venetoclax has shown promising activity in different combination therapies in a wide range of AML subtypes. Novel immunotherapy approaches are currently developed based on bispecific antibodies and as CAR T cell approaches but problems remain.

An important task for the coming time is to integrate the novel approaches into established treatment pathways including allogenic transplantation. Fortunately, it appears possible that outcome of AML may improve in the next years.
Conflict of Interest: Research funding from Pfizer, Janssen and Celgene. Advisory board and speaker's fees: Pfizer

LATE SUBMISSIONS

Progress in AML: The Future

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Acute myeloid leukemia (AML) is a phenotypically and prognostically heterogeneous hematopoietic stem cell disease which has mainly been treated with conventional chemotherapy and allogeneic stem cell transplantation so far. During the last two decades tremendous advances in molecular techniques, in particular massive parallel sequencing, have facilitated the discovery of a large number of molecular aberrations in patients with AML. This comprehensive view on the molecular landscape has substantially moved forward our understanding of the pathophysiology of AML, enables a better classification and risk estimation and allows the submicroscopic tracking of the disease (e. g. measurable residual disease monitoring). Furthermore, the insights into the underlying molecular AML biology have initiated the development of targeted therapies specifically tackling these molecular alterations. After decades without substantial progress in chemotherapy-based AML therapy this has resulted in the approval of the FLT3 inhibitor Midostaurin as first targeted therapy integrated into the first-line therapy of younger patients with FLT3-mutated AML. Besides second-generation FLT3 inhibitors (Gilteritinib, Quizartinib, Crenolanib) new therapies targeting molecular aberrations (e. g. IDH inhibitors), anti-apoptotic pathways (e. g. BCL inhibitors), but also surface antigens on leukemic cells (e. g. gemtuzumab ozogamicin) and new pharmacokinetic compositions of classical cytostatic drugs (e. g. CPX-351) or hypomethylating agents (e. g. CC-486) have entered the therapeutic arena or will be soon available. While initially often tested or approved in second or later lines of therapy many of these candidates are also under investigation in the front-line setting or in combination with chemotherapy or hypomethylating agents. Furthermore, in particular those drugs specifically targeting a molecular alteration are interesting candidates for maintenance approaches. The challenge will be to use this opportunity of new available drugs by integrating them into the current treatment algorithms in order to offer a more personalized therapy to AML patients tailored on the basis of the molecular information.

FREE CONTRIBUTIONS
Late Breaking Abstract

Outcomes Following Hematopoietic Stem Cell Transplantation in Patients Treated with Chemotherapy with or without Gemtuzumab Ozogamicin for Acute Myeloid Leukemia

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Background: The phase 3 ALFA-0701 study showed improved outcomes with the addition of gemtuzumab ozogamicin (GO) to standard 7+3 chemotherapy in patients with de novo acute myeloid leukemia (AML). We describe transplant characteristics and examine outcomes in patients who received hematopoietic stem cell transplantation (HSCT) on study.
Patients and method: Patients aged 50–70 y with *de novo* AML who received chemotherapy (daunorubicin and cytarabine) with GO or alone (control) and underwent HSCT in the ALFA-0701 study were analyzed. HSCT was recommended for those in first complete remission (CR1) with non core binding factor or normal karyotype and *NPM1+/FLT3-ITDwt* or *CEBPA* AML and with matched related or unrelated donor. In all, 85 patients (n=32 vs 53, GO vs control) received HSCT; 1 patient in the control arm received autologous HSCT and all others allogeneic HSCT. Patients in the GO vs control arm received HSCT in CR1 (n=17 [53%] vs 22 [42%]), after relapse (n=13 [41%] vs 22 [42%]), and after induction failure (n=2 [6%] vs 9 [17%]). One patient in the control arm who received GO as follow-up therapy underwent HSCT <2 mo after last GO dose.
Results: In the GO vs control arm, median post-transplant survival was 21.4 vs 17.1 mo (hazard ratio [HR] 0.97) and 3-y survival probability (95% confidence interval [CI]) 39% (22–57) vs 45% (31–58); at 12 mo, cumulative incidence rate of relapse (95% CI) was 9% (2–23) vs 31% (19–44; HR 0.60) and cumulative incidence rate of non-relapse mortality (95% CI) was 29% (14–45) vs 22% (12–34; HR 1.69). Veno-occlusive disease (VOD) was reported in 5 patients, 3 in the GO arm vs 2 in the control arm (both received GO as follow-up therapy); 3 events (n=2 vs 1) occurred after HSCT. All but 1 patient fully recovered. Similar post-transplant outcomes were observed in patients with AML treated with chemotherapy alone or with GO. GO was not associated with an excess of VOD events after HSCT.
Conclusion: Results suggest GO as part of induction and consolidation chemotherapy for AML does not induce excess post-transplant mortality and thus does not preclude the use of HSCT as consolidation treatment following induction or salvage treatment.

SATELLITE SYMPOSIA

EVENING SYMPOSIUM
(Vector Therapeutics, Inc.)

Immunotherapy for Relapse Prevention in Acute Myeloid Leukemia
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Relapse after the completion of chemotherapy remains a therapeutic challenge in acute myeloid leukemia (AML) and novel strategies aiming to delay or prevent relapse are highly warranted. In the post-chemotherapy phase of AML, functions of natural killer (NK) cells and cytotoxic T cells have been ascribed a protective role, which implies that activation of NK and T cell function may decrease the risk of relapse. Several immunotherapies, including cytokines, dendritic cell vaccines, multispecific antibodies and strategies to target the PD-1/PD-L axis are currently evaluated for relapse prevention. At this symposium, sponsored by Vector Therapeutics, the prospect of utilizing immunotherapy for maintenance of complete remission will be discussed with focus on the use of histamine dihydrochloride and low-dose interleukin-2 (HDC/IL-2). This combinatorial immunotherapy promotes NK cell and T cell function and was previously shown to reduce relapse risk in a phase III trial (Brune *et al*., *Blood* 2008; Martner *et al*., *Blood Rev* 2013). We will present data on the anti-leukemic efficacy of the HDC component in experimental *in vivo* models of myeloid leukemia (Aydin *et al*., *Oncogene* 2018). These results suggest that HDC targets the immunosuppressive NOX2 enzyme expressed by normal and leukemic myeloid cells (Martner *et al*., *J Pathol* 2018). We will also present phase IV trial results using HDC/IL-2 addressing the role of cell-mediated immunity in preventing relapse of AML (Martner *et al*., *Oncoimmunology* 2015; Bernson *et al*., *Leukemia* 2017; Hallner *et al*., *Blood* 2019) along with novel clinical data on the efficacy of HDC/IL-2 in defined molecular subgroups of AML.

SATELLITE SYMPOSIUM II
(AbbVie Deutschland GmbH)

Welcome and Introduction
Uwe Platzbecker
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Currently, the choice of therapy for AML is driven by the patient's suitability for intense chemotherapy. Low-intensity options are typically preferred for older or unfit patients, but response rates are low and median overall survival remains at less than 1 year. In this symposium, we will review the potential impact of novel agents, explore possible strategies to tackle the emergence of resistance to novel agents, and consider expert opinion for the management of AML therapy in older or frail populations.

Emerging Agents for the Treatment of Patients with AML
Uwe Platzbecker

Universitätsklinikum Leipzig, Hämatologie und Zelltherapie, Leipzig, Germany

Although many promising novel agents for the treatment of AML have been evaluated over the past few decades, almost all have proved unsuccessful in improving outcomes for patients with this disease. However, more-recent advances in the understanding of the genetics of AML have translated into the development of new targeted drugs against AML driver mutations such as mutant isocitrate dehydrogenase (*IDH*) and FMS-related tyrosine kinase 3 (*FLT3*). Drugs targeting these agents are now becoming available for patients with AML found to be harboring these genetic changes. In addition, a better understanding of the molecular biology of AML has highlighted dysregulated pathways of importance for AML cell survival and resistance to treatment. Such pathways include the apoptotic pathway and the hedgehog signaling pathway, both of which can now be targeted with emerging novel therapeutics. These treatments may offer additional possibilities for patients who are ineligible for intensive chemotherapy and would previously have had more-limited treatment options.

From Bench to Bedside: Practical Considerations for the Older Patient with AML

Uwe Platzbecker
Universitätsklinikum Leipzig, Hämatologie und Zelltherapie, Leipzig, Germany

In the wake of emerging targeted agents for the treatment of older patients with AML, expert opinion will consider treatment management for this specific population, which frequently has a high number of associated comorbidities. The management of adverse events related to novel targeted agents and practical considerations for patient compliance will also be covered.

Targeting Apoptosis in AML

Marina Konopleva
MD Anderson Cancer Center, The University of Texas Health Science Center at Houston and The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Evasion of apoptosis is a hallmark of malignant cell survival. AML cells often evade apoptosis via dependence on BCL-2 and other pro-survival proteins, such as MCL-1 and BCL-X_L. Several small-molecule inhibitors of pro-survival proteins are currently being explored in the clinic. Venetoclax, a highly selective BCL-2 inhibitor, has recently been granted accelerated approval by the FDA in combination with hypomethylating agents (HMAs; azacitidine or decitabine) or low-dose cytarabine (LDAC) in untreated patients who are ineligible for intensive chemotherapy due to age or comorbidities. These combinations are well tolerable and elicit high response rates and durable responses in a subset of elderly newly diagnosed AML patients.

Strategies to Overcome Resistance to Emerging Agents

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As potent new targeted therapies are changing the landscape of AML treatment, new resistance mechanisms are being identified. A better understanding of the underlying mutations and resistance pathways is required to optimize the use of these novel agents. Combination regimens (including, for example, FLT3 inhibitors, CDK9 inhibitors, BCL-2 inhibitors, MCL-1 inhibitors, ± high-dose chemotherapy) are attractive strategies to tackle the development of resistance and eliminate resistant clones. Identification of biomarkers predicting treatment sensitivity/resistance will also help lead the way to more-personalized medicine.

SATELLITE SYMPOSIUM V
(Roche Pharma GmbH)

MDM2 Inhibition as a New Treatment Approach in AML

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Idasanutlin is a potent oral inhibitor of MDM2 currently being explored in clinical trials. MDM2 regulates stability of wt-TP53, and inhibition of MDM2 was shown to cause p53-mediated cell-cycle arrest and/or apoptosis in cancer cell lines (*Tovar et al., PNAS 2006*). Furthermore, combined inhibition of MDM2 and BCL-2 was shown to be synergistic in preclinical AML models (*Pan et al., Cancer Cell 2018*). In a phase I/Ib study in relapsed/refractory (R/R) AML idasanutlin was generally well tolerated, and idasanutlin + cytarabine induced a 29% rate of composite complete remission (CRc, CR + CRp + CRi) with a median duration of response (mDoR) >8 months (*Martinelli et al. EHA 2016, abstract S504).* A randomized phase III study (MIRROS) of intermediate doses of cytarabine with or without idasanutlin is ongoing. In an ongoing phase Ib study of idasanutlin combined with the BCL-2 inhibitor venetoclax, the combination has demonstrated a tolerable safety profile and induced a 33% CRc rate in R/R elderly AML; confirmation of the recommended phase 2 dose followed by expansion is planned (*Daver et al. ASH 2018, abstract 767*). Finally, future trials in frontline

untreated AML with idasanutlin added to 7+3 induction chemotherapy and/or given as maintenance will be discussed.