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Virtual Event, 15 – 17 March 2021

ABSTRACT BOOK

Hematology Reports 2021; 13:s1

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ABSTRACT BOOK

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BIOLOGY OF ACUTE LEUKEMIAS

01

INTRACELLULAR AND BIOFLUID METABOLOMICS DISTINGUISHES TWO SUBGROUPS OF NPM1-MUTANT ACUTE MYELOID LEUKEMIA WITH DIVERSE GENOMIC AND TRANSCRIPTOMIC SIGNATURES

G. Simonetti[1], C. Mengucci[2,3], A. Padella[1], E. Fonzi[1], G. Picone[2], C. Delpino[4], J. Nanni[5,6], R. De Tommaso[6], E. Franchini[1], C. Papayannidis[5], G. Marconi[1], M. Pazzaglia[6], M. Perricone[6], E. Scarpi1, M.C. Fontana[1], S. Bruno[5,6], M. Tebaldi[1], A. Ferrari[1], M.T. Bochicchio[1], A. Ghelli Luserna di Rorà[1], M. Ghetti[1], R. Napolitano[1], A. Astolfi[7], C. Baldazzi[5], V. Guadagnuolo[6], E. Ottaviani[5], I. Iacobucci[8], M. Cavo[5,6], G. Castellani[6], T. Haferlach[9], D. Remondini[3], F. Capozzi[2], G. Martinelli[1]

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Introduction: Genomic and functional alterations of enzymatic activity drive cancer metabolic reprogramming along with mutations of tumor suppressors and oncogenes. Although targeting of cell metabolism is a promising therapeutic strategy in acute myeloid leukemia (AML), metabolic dependencies are largely unexplored. We aimed to classify AML patients based on their metabolic landscape and map connections between their metabolic and genomic profiles.

Methods: Genomic data of AML patients were obtained by whole exome sequencing (n=160) or targeted NGS (n=21). Of them, 119 were evaluated for biofluids (serum and urine) metabolites by nuclear magnetic resonance, along with 145 healthy controls. Intracellular metabolites of AML bone marrow (BM) cells (n=50) and controls (n=42) were analyzed by mass spectrometry. RNA-seq data of NPM1-mutated (mut) AML were obtained from TCGA (n= 66) and BEAT-AML (n= 121) cohorts.

Results: Mutations of metabolic enzymes and regulators were detected in 88% of patients. Unsupervised hierarchical clustering of AML based on the intracellular metabolome distinguished three clusters, characterized by enrichment of distinct genomic profiles: *NPM1*-mut, with high metabolite concentration, chromatin/spliceosome-mut, with low metabolite levels and *TP53*/aneuploid AML, with a more complex metabolic profile. The clusters were also observed at biofluid level and had an opposite trend of tyrosine and threonine level between serum and blasts. Interestingly, integrated genomic-metabolic profiles defined two subgroups of *NPM1*-mut AML. One was enriched for mutations in cohesin/DNA damage-related genes and showed increased serum choline+trimethylamine-N-oxide and leucine, higher mutation load, transcriptomic signatures of reduced inflammatory status, better *ex-vivo* response to EGFR and MET inhibition and differential expression of 7 genes mapping on the human metabolic network. In silico modelling of the intracellular metabolic perturbations induced by these genes predicted alterations in the purine and NAD metabolic superpathways in this subgroup of *NPM1*-mut AML, that were supported by intracellular metabolic data.

Conclusions: AML is characterized by genomic-driven and functional metabolic alterations. The integration of genomic and metabolic profiles suggests potential vulnerabilities worthy of being therapeutically explored in a subgroup of *NPM1*-mut AML.

Supported by: EHA Research Fellowship award, AIRC, FP7-NGS-PTL, Fondazione del Monte, Haferlach Leukämiediagnostik Stiftung, Ministry of Health RRC-2020-23670007 (ERAP-ERMED2019-290).

02

MTORC INHIBITION DECREASE G6PD LEVELS AND TRIGGERS CELL DEATH IN T-LYMPHOBLASTIC LEUKAEMIA CELLS

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Background: Approximately 25% of patients with pediatric T-cell acute lymphoblastic leukaemia (T-ALL) are incurable at present. We previously showed that T-ALL cells exhibit high levels of reactive oxygen species (ROS). We showed that altering redox homeostasis primed T-ALL cells to apoptosis triggered by the glucocorticoid Dexamethasone and indicated the reliance of T-ALL cells on the activity of the pentose phosphate pathway (PPP).

Here we investigated the mechanism underlying this rewiring of redox homeostasis and the possible involvement of the mTOR pathway, which is constitutively activated in a high proportion of T-ALL. The present study investigated the impact of the mTOR pathway on ROS homeostasis and viability of glucocorticoid-resistant T-ALL cells.

Methods: T-ALL cells were treated with the mTORC1 inhibitor Everolimus. Effects on gene expression were tested by quantitative RT-PCR and immunoblotting. Effects on ROS and cell death were assayed using fluorescent probes and flow cytometry. *In vivo* effects were evaluated in mice inoculated with patient-derived T-ALL xenografts (PDX).

Results: Everolimus reduced the levels of the PPP enzyme glucose-6-phosphate dehydrogenase (G6PD) and NADPH, increased reactive oxygen species (ROS), and killed T-ALL cell lines, cells from patient-derived T-ALL xenografts (PDX) and primary tumor cells from T-ALL patients, while sparing normal T-cells. These effects were blunted by ROS scavengers, and were phenocopied by siRNA-mediated silencing of the mTORC1 cofactor Raptor and G6PD. *In vivo* studies of NOD/SCID mice inoculated with PDX from glucocorticoid-refractory T-ALL cells confirmed that Everolimus overcame Dexamethasone resistance.

Conclusions: These findings provide insight into the crosstalk between mTORC1 and ROS homeostasis in T-ALL and suggest the combination of glucocorticoids with mTORC1 inhibitors as a therapeutic avenue to target refractory T-ALL.

03

PHARMACOLOGICAL INHIBITION OF WIP1 SENSITIZES ACUTE MYELOID LEUKEMIA CELLS TO THE MDM2 INHIBITOR NUTLIN-3A

M.C. Fontana[1-2], J. Nanni[3], A. Ghelli Luserna di Rorà[1], E. Petracci [1], A. Padella[1], M. Ghetti[1], A. Ferrari[1], G. Marconi[1], S. Soverini[2], I. Iacobucci[4], C. Papayannidis[5], A. Curti[5], E. Audisio[6], M.B. Giannini[1], M. Rondoni[7], F. Lanza[7], M. Cavo[3-5], G. Martinelli[1], G. Simonetti[1]

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Introduction: In acute myeloid leukemia (AML), the restoration of p53 activity through MDM2 inhibition proved efficacy in combinatorial therapies. WIP1, encoded from PPM1D, is a negative regulator of p53. We here evaluated PPM1D expression and explored the therapeutic efficacy of WIP1 inhibitor (WIP1i) GSK2830371, in association with the MDM2 inhibitor Nutlin-3a (Nut-3a) in AML cell lines and primary samples.

Methods: PPM1D expression and its correlation with clinical and molecular data was analyzed in available transcriptomic dataset GSE6891, GSE13159 and TCGA. *In vitro* viability and apoptosis assays after treatment was evaluated on TP53-wt (MOLM-13, MV-4-11, OCI-AML3) and TP53-mut (NOMO-1, HEL, KASUMI-1) AML cell lines and on 9 AML patients. Gene expression profiling (GEP) and immunoblotting analyses were performed after 16h of treatment.

Results: PPM1D transcript levels were higher in young patients compared with older ones and in core-binding-factor AML compared with other cytogenetic subgroups. Its expression was reduced in NPM1-mutated (irrespective of FLT3-ITD status) or TP53-mut cases compared with wt-ones. *In vitro* single agent Nut-3a (0.5, 1, 2.5, 5 uM) did not reduce cell viability in the TP53-mut cells, while showing a time and dosage-dependent effect in all the TP53-wt cells, with IC50 values below 1 μM at 72h. WIP1i as single agent (5, 10, 20 uM) did not significantly affected MOLM-13, HEL, KASUMI-1 and NOMO-1 cell viability. The combined treatment reduced the viability of TP53-wt cells, while sparing the TP53-mut ones. The combination index analyses showed a synergic effect of drugs combination in TP53-wt cells, especially using low Nut-3a doses. A significant increase in the percentage of apoptotic cells in the TP53-wt cell lines at 48h was detected. In line with the cell viability results, apoptosis of TP53-mut cells were barely affected by the combined treatment. These results were confirmed in ex vivo assays of primary TP53-wt AML leukemic cells, where the combined treatment induced a significant decrease of cell viability and a progressive increase of apoptotic cells. To elucidate changes in the transcriptional program of AML cells induced by the combination, we performed GEP was performed on representative TP53-wt and TP53-mut cell lines (MV4-11 and NOMO1), showing enrichment of a p53 signature in MV-4-11 cells treated with the drug combination upregulating in particular MDM2, CDKN1A. The protein level p53 and of key signature genes was evaluated in the whole panel of cell lines, where the upregulation of p53, p21 and MDM2 was confirmed in TP53-wt cells.

The p53 pathway was not affected in the TP53-mut cell lines. GSEA analyses highlighted the enrichment of MYC, PI3K-AKT-mTOR and inflammation-related signatures upon WIP1i, Nut-3a and their combination, respectively, in the MV-4-11 TP53-wt model.

Conclusions: Overall, these data indicate that WIP1 is a promising therapeutic target to enhance Nut-3a efficacy in TP53-wt AML cells.

04

UNDERSTANDING THE ROLE OF THE ATR-CHK1 PATHWAY IN THE RESPONSE TO DOXORUBICIN-INDUCED DNA DAMAGES IN ACUTE LYMPHOBLASTIC LEUKEMIA

A. Ghelli Luserna Di Rorà[1], M. Ghetti[1], L. Ledda1, A. Ferrari[1], M. Bocconcelli2, A. Padella[1], R. Napolitano[1], M.C. Fontana[1], C. Liverani[1], E. Imbrogno[1], M.T. Bochicchio[1], M. Paganelli[1], V. Robustelli[2], S. Sanogo[3], C. Cerchione[1], G. Musuraca[1], G. Martinelli[1], G. Simonetti[1]

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Background: Doxorubicin is one of the most commonly used anthracyclines for the treatment of solid and hematological tumors such as B-/T-cell acute lymphoblastic leukemia (ALL). Doxorubicin (Dox) compromises topoisomerase-II enzyme functionality inducing structural damages during DNA replication (replication stress). In particular, Dox causes DNA double strand breaks (DSBs) the most cytotoxic form of DNA damages. Eukaryotic cells respond to DSBs activating ATM-CHK2 and/or ATR-CHK1 pathway whose function is to regulate cell cycle progression and promote damage repair.

Methods: Human B-/T- cell precursor ALL cell lines and primary leukemic cells isolated from adult ALL patients were treated with Dox followed by specific ATR (VE-821) or CHK1 (prexasertib, PX) inhibitors. The efficacy of the drug schedule was evaluated in term of reduction of cell viability, induction of apoptosis, cell cycle profile and induction of DNA damages (immunoblotting, immunofluorescence and chromosome integrity assays).

Results: ALL cell lines respond to Dox activating the G2/M cell cycle checkpoint. We found that the treatment with VE-821 and PX in Dox-pretreated ALL cell lines, enhance the cytotoxicity of Dox. This phenomenon is related to the abrogation of the G2/M cell cycle checkpoint, the entry in mitosis followed by the induction of apoptosis. Indeed, the inhibition of the G2/M checkpoint led to a significant increment of normal and aberrant mitotic cells. In particular, we found that the inhibition of ATR or CHK1 kinases in Dox pre-treated ALL generated cells with tripolar spindles, with micro/macro nuclei, metaphases with lagging chromosomes and massive chromosome fragmentation.

Conclusions: The ATR-CHK1 pathway is involved in the response to Dox-induced DNA damages. Our data suggests that novel therapeutic strategies combining ATR-CHK1 inhibitors with Dox might be an effective anti-leukemic strategy for the treatment of ALL patients.

05

EXPLORING PVT1/CIRCPVT1 ROLES AND THEIR REGULATION THROUGH BROMODOMAIN AND EXTRA-TERMINAL MOTIF PROTEIN INHIBITORS IN ACUTE MYELOID LEUKEMIA

M. Ghetti[1], A. Padella[1], I. Vannini[1], S. Bruno[2], C. Liverani[1], L. Montanaro[2], A. Ghelli Luserna di Rorà[1], M.C. Fontana[1], C.T. Storlazzi[3], G. Martinelli[1], G. Simonetti[1]

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Introduction: Long-non-coding RNAs, including circular (circ)RNAs, are involved in cancer progression. The human Plasmacytoma Variant Translocation 1 (PVT1) gene maps on chromosome 8, encodes for 83 linear (PVT1) and 25 high-confidence circular isoforms (circPVT1) and plays MYC-dependent and independent functions. In acute myeloid leukemia (AML), PVT1 is highly expressed in high-risk cases from t(15;17), t(8;21) subtypes (Gómez-Seguí, PLoS One 2014; El-Khazragy, Gene 2019) and in acute erythroleukemia (Houshmand, Int J Bioch Cell Biol 2018). Bromodomain and extra-terminal motif-protein inhibitors (BETi) have shown an antileukemic effect in several subtypes of AML, both *in vitro* and *in vivo* (Daniel Gerlach, Oncogene 2018). The present study aims to define the role of circPVT1 and PVT1 isoforms in leukemogenesis and to investigate the role of BETi in the regulation of the circular and linear forms of PVT1 in AML.

Methods: We studied the diverse PVT1 isoforms from the NONCODE database and their differential expression in AML. Then we designed antisense oligonucleotide ASOs targeting the isoforms expressed in OCI-AML3 cells. circPVT1 was silenced by siRNA in order to investigate the effects on apoptosis, proliferation, and its downstream targets in OCI-AML3 cells. Moreover OCI-AML3 and KASUMI-1 AML cell lines were treated for 16 hours with BETi under normoxic and hypoxic conditions. circPVT1 and PVT1 were quantified by qRT-PCR, using total RNA and di-polisomal fraction. The qRT-PCR results were also confirmed by RNA-ISH in combination with the S6 ribosomal marker.

Results: We focused on 9 PVT1 isoforms detected in lymph nodes and/or white blood cells and OCI-AML3 cell line. ASOs-mediated knockdown (KD) of PVT1 isoform blocks in OCI-AML3 showed a relevant decrease of signals from both regions by ASO combination. The downregulation led to a modest, though significant decrease in cell growth. Our data on circPVT1 silencing in OCI-AML3 cells showed reduced cell growth, a slight increase in apoptosis and no effects on MYC transcript. Moreover, the interferon (IFN)-inducible serine/threonine protein kinase PKR, which has been previously reported to have a role in the innate immunity and to be inhibited by circRNAs, was induced by circPVT1-silencing in leukemic cells, as observed in T lymphocytes from systemic lupus erythematosus. circPVT1 and PVT1 total RNA significantly decreased in both cell lines after treatment with BETi, under normoxia and hypoxia. circPVT1 and PVT1 were also detected in the polysomal fraction. Interestingly, PVT1 showed mostly a nuclear localization, while circPVT1 colocalized with ribosomes.

Conclusions: PVT1 isoforms and circPVT1 may contribute to AML pathogenesis and progression, and targeting them may have therapeutic potentials. Our findings show that circPVT1 and PVT1 are regulated by BETi, along with MYC, in AML cell lines under normoxia and hypoxia.

06

PALB2 COPY NUMBER LOSS ASSOCIATES WITH POOR PROGNOSIS AML AND SUGGESTS NOVEL THERAPEUTIC STRATEGIES TARGETING THE DNA REPAIR PATHWAY

A. Padella[1], M.C. Fontana[1], G. Marconi[2], E. Fonzi[1], E. Petracci[1], A. Ferrari[1], C. Baldazzi[2], C. Papayannidis[2], A. Ghelli Luserna Di Rorà[1], N. Testoni[2], G. Castellani[2], T. Haferlach[3], G. Martinelli[1], G. Simonetti[1]

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Partner and localizer of BRCA2 (PALB2) is a Fanconi Anemia gene and heterozygous germline mutations are associated with an increased risk of developing pancreatic, ovarian or breast cancers. Little is known of its role in acute myeloid leukemia (AML). Here, we described genomic alterations of PALB2 in a cohort of 233 AML patients genotyped with SNP array. More than 5% of AML cases carried heterozygous copy-number (CN) loss of PALB2 in our cohort. Patients with PALB2 CN loss were frequently affected by TP53 genomic mutations or CN alterations ($p<0.001$), they were older ($p=0.044$) and showed adverse prognosis ($p=0.016$). These findings were validated in two independent AML cohorts, where expression data confirmed that patients with lower expression levels of PALB2 were older and with poor prognosis ($p=0.007$ and $p\leq 0.021$, respectively). At clinical level, PALB2 CN loss had a negative effect on overall survival (OS, median 2.0 *vs* 16.2 months in PALB2 loss and wild-type, respectively; $p<0.0001$). Notably, TP53 status, age, risk and type of AML did not affect the prognostic effect of PALB2 on OS. Our data identified PALB2 as a potential biomarker for very-poor prognosis AML.

07

EPIGENETIC REGULATION OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive leukemia with a high incidence in children, adolescents and young adults. Although multiple therapeutic options are available, almost one fifth of patients affected by T-ALL eventually succumb to the disease, suggesting an unrecognized biological complexity that might contribute to drug resistance. To better understand the differences between T-ALL subtypes and their clinical course, we systematically analyzed cohorts of patients with different risk profiles at the genetic and epigenetic level. We recently demonstrated that differences in three-dimensional (3D) chromatin architecture can influence the integrity of topologically associating domains (TADs) and rewire specific enhancer–promoter interactions, impacting gene expression and leading to disease. As an example, we focused in particular on the Myc family of oncogenes, revealing disease-specific patterns of enhancer-promoter interactions.

Methods: We initially profiled a large cohort of T-ALL patients falling under different risk categories in order to identify differences in their genetic and epigenetic features. We then systematically integrated matched in situ Hi-C, RNA-seq and CTCF ChIP–seq datasets to reveal widespread differences in intra-TAD chromatin interactions and TAD boundary insulation in patients affected by T-ALL.

Results: Using primary acute leukemia patient samples, we have, for the first time, identified recurrent TAD disruptions in leukemia involving key oncogenes (e.g. NOTCH1, c-Myc) and their targets. For example, we identified a recurrent disruption of 3D chromatin topology in the c-Myc locus at a previously uncharacterized non-coding CTCF-bound region that insulates MYC from a downstream super-enhancer. This disruption enables chromatin interactions between the c-Myc oncogene and the downstream super-enhancer leading to an increase in c-Myc expression.

In parallel, while focusing patients falling into a higher risk category, namely the early T cell progenitor acute lymphoblastic leukemia (ETP-ALL), we discovered a previously uncharacterized region of high activity encompassing a novel lncRNA interacting with the proto-oncogene N-Myc.

Conclusions: With the current study we demonstrated an inherent difference between subtypes of T-ALL based on their epigenetic profile, which in turn influences the expression of key oncogenes. By focusing on a new methods of regulating a known family of transcription factors, we provide a new mechanism which could open interesting ways for targeted therapy of patients at different risk levels.

08

PERSONALIZED SURVIVAL PREDICTION OF PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA USING GENE EXPRESSION PROFILING

A. Mosquera Orgueira[1,2,3*], A. Peleteiro Raíndo[1,2,3*], M. Cid López[1,2,3], J.Á. Díaz Arías[1,2], M.S. González Pérez[1,2], B. Antelo Rodríguez[1,2,3], N. Alonso Vence[1,2,3], L. Bao Pérez[1,2,3], R. Ferreiro Ferro[1,2], M. Albors Ferreiro[1,2], A. Abuin Blanco[1,2], E. Fontanes Trabazo[1,2], C. Cerchione[4], G. Martinelli[4], P. Montesinos Fernández[5], M. Mateo Pérez Encinas[1,2,3], J.L. Bello López[1,2,3]
**These authors contributed equally as first co-authors.*

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Introduction: Acute Myeloid Leukemia (AML) is a heterogeneous neoplasm characterized by cytogenetic and molecular alterations that drive patient prognosis. Currently established risk stratification guidelines show a moderate predictive accuracy, and newer tools that integrate multiple molecular variables have proven to provide better results.

Methods: In this report, we aimed to create a new machine learning model of AML survival using gene expression data. We used gene expression data from two publicly available cohorts in order to create and validate a random forest predictor of survival, which we named ST-123.

Results: The most important variables in the model were age and the expression of KDM5B and LAPTM4B, two genes previously associated with the biology and prognostication of myeloid neoplasms. This classifier achieved high concordance indexes in the training and validation sets (0.7228 and 0.6988, respectively), and predictions were particularly accurate in patients at the highest risk of death. Additionally, ST-123 provided significant prognostic improvements in patients with high-risk mutations.

Conclusions: Our results indicate that survival of patients with AML can be predicted to a great extent by applying machine learning tools to transcriptomic data, and that such predictions are particularly precise among patients with high-risk mutations.

CLINICAL OF ACUTE LEUKEMIAS

09

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS OLDER THAN 65 YEARS WITH AML AND MDS: A 15-YEAR MONOCENTRIC EXPERIENCE

S. Piemontese, L. Lazzari, M. Marcatti, A. Ruggeri, M.T. Lupo Stanghellini, F. Giglio, R. Greco, F. Lorentino, D. Clerici, A. Assanelli, F. Farina, S.Mastaglio, E. Xue, S. Marktel, C. Corti, M. Carrabba, M. Bernardi, J. Peccatori, F. Ciceri

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Introduction: Allogeneic stem cell transplant (allo-HCT) has been historically limited to younger population, due to excess in non-relapse-mortality (NRM) and relapse in elderlies. Here we report our 15-year experience in allo-HCT in patient older than 65 years diagnosed with AML or MDS.

Methods: All consecutive patients aged more than 65 years and receiving an allo-HCT (as first transplant) for AML or MDS at our center from January 2005 to December 2019 were included in this study.

Results: Ninety patients fulfilled the inclusions criteria (71, 81% had AML and 19, 19%, MDS). Median age was 68.29y (65.02-76.54), with 29% being older than 70 years. Donors were HLA-matched related in 13pts (14%), matched unrelated (10/10) in 20pts (22%), mismatched unrelated (9/10) in 13pts (14%) and haploidentical in 44pts (50%). In 78pts (87%) conditioning was treosulfan-based. Fourty-six (51%) patients received a myeloablative conditioning regimen. Eighty-five (94%) pts received in-vivo T-cell depletion: in 54 (63%) with anti thymocyte globulin (ATG) and in 31 (37%) with post-transplant cyclophosphamide (PTCy). Fourty-four (49%) pts had a HCT-CI of 0, 31 (34%) pts of 1 or 2 and 15 (17%) pts ≥ 3. Disease Index Risk was low for 1 (1%) pt, intermediate for 39 (44%) pts, high for 42 (48%) pts and very high for 7 (8%) pts (not available in 1 pt). Median follow-up was 35.08mo (2.82-104). Median duration of hospitalization was 48 days (24-198). The 3-year overall survival (OS) was 53+/-6% and disease free survival (DFS) 45+/-6% (Figure 1).

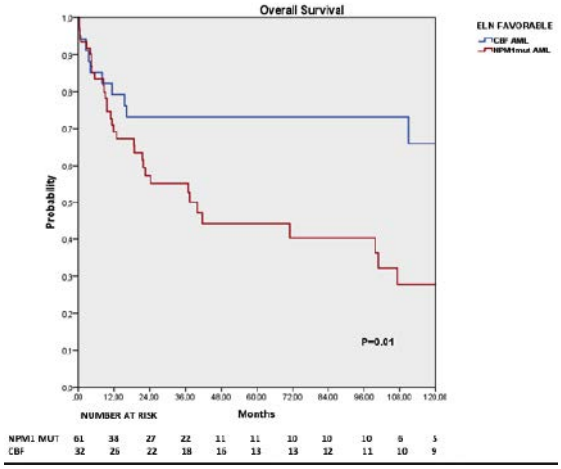

Figure 1.

Day-100 and 3-year NRM was 17+/-2% and 29+/-2%, respectively. The 3-year CI of relapse was 22+/-2%. Day-100 CI of aGvHD was 21+/-2% for grade II-IV, 14+/-1% for grade III-IV. The 3-year CI of cGvHD was 35+/-3%, extensive 20+/-2%. In multivariate analysis Karnofsky Performance Status (KPS) < 90% was associated with lower OS (HR: 2.999, CI: 1.477- 6.691; p=0.002), lower DFS (HR: 3.155, CI: 1.593 - 6.250; p=0.001) and higher NRM (HR: 2.997, CI: 1.344-6.682; p=0.041). HCT-CI ≥ 3 was another risk factor for higher NRM (HR: 2.949, CI: 1.166-7.462, p=0.022,). Diagnosis of MDS and having received a matched donor with PTCy were risk factors for longer OS (HR: 0.3440, CI: 0.1029-0.915; p=0.033; HR: 0.197, CI: 0.042-0.934; p=0.041).

Conclusions: Age alone should not limit allo-HCT eligibility for AML and MDS patients. KPS and HCT-CI confirm to be useful for patient selection among elderlies. The use of HLA-matched donors with PTCy improved OS compared to ATG.

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GOOD RISK AML, A HETEROGENEOUS GROUP: ANALYSIS OF OUTCOME ACCORDING TO MOLECULAR SUBGROUPS

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Introduction: ELN-2017 prognostic model (Dohner et al, 2017) represents a a critical tool for risk stratification in AML patients. NPM1-mut AML has been historically associated with higher rates of remission and longer survival compared to other AML subtypes. However, prognosis depends strongly on the presence of co-occurring mutations such as FLT3-ITD. Further, recent evidence suggests that the prognostic role of NPM1 mutation in several subsets of patients is still controversial as it may depend on a variety of accompanying mutations other than FLT3-ITD. Moreover, the favorable prognostic impact of NPM1 seems to diminish in elderly patients.

Methods: We conducted a retrospective analysis, including all consecutive patients diagnosed with AML at Hematology Clinic, OspedaliRiuniti Ancona, between 1997 and 2020. AML was diagnosed according to the current WHO criteria. When cryopreserved sample was available, retrospective genetic analysis, including FLT3-ITD allelic-ratio assessment, were performed. Patients were stratified according to ELN 2017 recommendations. For time-to-event analysis, we calculated survival estimates using the Kaplan–Meier method and compared groups by the log-rank test.

Results: 608 patients were included in the analysis. Four hundred-twenty-eight patients underwent intensive chemotherapy. According to the ELN-2017 recommendations, they were classified as follows: 95 patients favorable risk; 172 patients intermediate risk; 125 patients adverse risk. Thirty-six patients had no evaluable samples for genetics and were excluded from outcome analysis. Within the favourable group, 53 patients had NPMmut-FLTwt AML, 8 NPMmut-FLTlow AML, 2 biallelic CEBPa-mut AML , 32 with CBF-AML, respectively. NPM1mut-FLT3-ITDwt AML and NPM1mut-FLT3-ITDlow AML had no significant difference in terms of OS (p=0.62) and LFS (p=0.76), while NPM1-mutated AML had a significantly lower OS (p<0.01) and LFS (p<0.01) as compared to CBF AML.

Conclusions: Our study demonstrated that NPM1-mut AML, independently from FLT3-ITD mutational status/allelic ratio, had a significantly worse outcome compared to CBF AML, despite sharing the same ELN 2017 risk class. This result suggests that NPM1-

mut AML has not an estabilished favorable outcome per se, but rather the favorable prognostic impact of NPM1 mutation is context-dependent. Assessment of co-occurring mutations by NGS might be of particular importance. A multi-center project is ongoing, with the aim of confirming the results of the present study on a larger cohort of patients with AML.

11

PRODUCTION OF ANTI CD19 CAR-T CELLS AND RATIONAL DESIGN OF A TRIAL TESTING ANTI CD19 CAR-T CELL EFFECTIVENESS IN ADULTS WITH CD19 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN COMPLETE REMISSION WITH MEASURABLE RESIDUAL DISEASE

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There is a persistent need for development of new and effective therapies for treatment of acute lymphoblastic leukemia, and to optimize the available ones. In early phase studies, chimeric antigen receptor (CAR)-T cells have demonstrated activity against ALL. Therapy related toxicities and lack of efficacy represent a problem addressed with various research attempts. Nowadays, in Italy, there is a diffuse limitation in using CAR-T cells for research purposes or out of commercial indication, due to technological delay, economic impact on national health system and cost/benefit limitations in a poorly funded research system. Commercial CAR-T cell therapy is available only for relapse/refractory young ALL patients, in a limited number of center and with limited slots. For this reason, we plan to set up a facility to produce CAR-T cell to address unmet clinical needs that are not covered by commercially available products.

We plan to establish and optimize production, working together with regulatory specialists and biology specialists to obtain a GCP graded anti CD19 CAR-T cell. The viral vector will be provided by Milteni biotech free of any cost for this research. In 20 months, we expect to achieve the main milestone, having a cellular product that can be infused in human in a clinical trial.

The second milestone will be to find strategies to implement CAR-T safety and CAR-T clinical efficacy. This will include - but will not be limited to - the *in vitro* testing of synergism with BTK inhibitors, the tests of performance of fresh *vs* frozen CAR-T cells, differences at single-cell level, to test the role of monocyte in cytokine release and the effect of monocyte inhibition and depletion also in animal models.

Contemporarily, we will start to design a clinical trial that will test the safety and the activity of our CD19 CAR-T cells against MRD positive CD19 positive ALL patients and that will incorporate all the advancements that will come from the laboratory.

The final achievement will come with the clinical trial results, that will give a preliminary test of CR19 CAR-T effectiveness on MRD positive ALL patients.

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DEVELOPMENT OF A NOVEL PHARMACOLOGICAL STRATEGY TO OVERCOME RESISTANCE TO ABT-199 IN ACUTE MYELOID LEUKEMIA (AML)

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Introduction: AML is a neoplasm of immature myeloid cells, with a ~20% 5-year survival rate. Although ~70% of adults with AML attain complete remission after induction therapy, disease relapse is common and often fatal, and only a restricted subset of patients is eligible for bone marrow transplantation. Novel therapeutic options are urgently needed to improve the outcome of these patients. A key determinant of AML chemoresistance is the overexpression of the anti-apoptotic protein BCL-2, suggesting that BCL-2 inhibitors could be effective to treat these patients. Consistently, ABT-199, an inhibitor of BCL-2, has received FDA approval for the treatment of AML, although in most patients leukemia cells become resistant to this compound, underscoring the need for the development of strategies aimed at overcoming this resistance. Our previous findings indicated that full activation of the intrinsic apoptotic pathway requires mitochondrial priming by reactive oxygen species (ROS), suggesting that rewiring redox homeostasis in leukemia cells could overcome their resistance to ABT-199.

Methods: The response to ABT-199 (alone or in combination with "A", a drug that blunts ROS scavenging) was assessed in a panel of 6 AML cell lines through the evaluation of cell death, measured as propidium iodide exclusion in flow cytometry. Mitochondrial ROS accumulation was measured using MitoSOX Red superoxide indicator in flow cytometry. The synergistic effect on cell death of "A" and ABT-199 was measured using the Chou-Talalay method. Combination index was calculated using the CompuSyn software.

Results: We demonstrated that AML cell lines exhibited different degrees of resistance to ABT-199. Interestingly, treatment with "A" significantly increased the sensitivity to ABT-199, with a mean 50-fold reduction in the 50% cytotoxic concentration (CC50). Based on their response to drug combination, we could group these lines as "sensitive-sensitizable", "resistant-sensitizable" and "resistant-non sensitizable". Notably, isobologram analysis revealed that the interaction between these compounds was highly synergistic. Importantly, "A" did not harm healthy PBMCs and did not increase the cytotoxic effect of ABT-199 in these normal cells, suggesting that this approach could dramatically improve its clinical efficacy and possibly reduce side effects by allowing a reduction of its dosage. We are currently validating the efficacy of our strategy in primary AML cells from patients enrolled in the INTHEMA study and we plan to establish AML patient-derived xenografts to test our approach *in vivo*.

Conclusions: Our results indicate that manipulation of redox homeostasis is a promising strategy to enhance the clinical efficacy of ABT-199 in AML and could increase the therapeutic window, allowing the selective elimination of resistant cancer cells using lower (and less toxic) doses of ABT-199 and reducing the important side effects connected to the treatment.

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A PRELIMINARY ANALYSIS OF FLAM (ITALIAN NON-INTERVENTIONAL MULTI-CENTER STUDY OF FLT3 MUTATED AML PATIENTS): FLT3 RECEPTOR GENE MUTATIONAL ANALYSIS AND FLT3 INHIBITORS ADMINISTRATION IN THE REAL-LIFE CLINICAL PRACTICE

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Introduction: FLT3 receptor gene has been reported to be mutated in about 30% of AML. However, the proper prognostic role of these mutations, how to properly monitor FLT3 mutated clones and manage FLT3 inhibitors (FLT3i) are only some of the open issues. FLAM is an observational study involving FLT3 positive AMLs to gain clinical and molecular data useful to ameliorate real-life physicians' management of this disease. Here we report the results of a preliminary analysis of the study.

Methods: The retrospective phase of FLAM multi-center observational study enrolled each AML patient treated in 33 participating Italian centers detected to carry a FLT3 since 2012. Clinical and molecular data were collected in accordance with GCP and Helsinki declaration in electronic case report forms.

Results: At data cut-off, 1st of July 2020, 289 patients with FLT3 mutation at diagnosis were enrolled in the retrospective phase of FLAM study, being evaluable in this analysis (Patients' characteristics - Table 1). Among the study population, 255/289 (88%) patients carried a FLT3-ITD, 32/289 (11%) a FLT3-TKD point mutation and 2/289 (1%) patients both. Capillary electrophoresis has been the technical method used to investigate FLT3-ITD in 163 of 226 (72%) patients with information on the method used, while Sanger Sequencing in 47 out of 226 (21%) patients and Next generation sequencing (NGS) in 16 out of 226 (7%) patients. FLT3-ITD allelic ratio was available in 62 of 257 (24%) ITD patients and was ≥ to 0.5 in 35/62 (56%) patients. A more frequent association between FLT3-ITD and normal karyotype and between FLT3-TKD and other cytogenetic alterations not conferring a favorable/adverse risk has been observed (p=0.045). During patients' follow-up, 19/289 (7%) patients affected by a FLT3 positive AML at diagnosis underwent a disease clonal evolution with a FLT3 negative AML progression or relapse. Regarding treatment options in FLT3-AML, in our cohort FLT3i were administered as first-line of therapy in only 36/289 (13%) patients, always in a combination, of which in 26/36 (72.2%) with intensive chemotherapy. On the other hand, FLT3i were administered as rescue therapy in 62/171 (36%) cases (47/62 single-agent and in 15/62 in combination) and as re-induction therapy in 22/80 (28%) cases (10/22 single-agent and 12/22 in combination). Overall, a FLT3i has been administered as single-agent 81 times, of which Gilteritinib was the most frequently used (56/81, 69,1%), followed by quizartinib (18/81, 22,2%). Lastly, nine out of 289 (3%) patients received a FLT3i as maintenance therapy after HSCT.

Conclusions: These data coming from a preliminary analysis portray the state of a large multi-center retrospective cohort of FLT3-positive AML patients treated in Italy between 2012 and 2020, including interesting insights regarding technical methods used to characterize the disease and the therapeutic scenario in which FLT3 inhibitors have been developed.

Table 1.

Characteristics at AML diagnnosis	N patients = 289
Age at diagnosis *Median, years (min-max)*	62 (18 - 94)
Sex, *N (%)*	
- M	141 (48.8 %)
- F	148 (51.2 %)
AML type, *N (%)*	
- *De novo*	255 (88.2%)
- Secondary	29 (10.0 %)
- Therapy-related	5 (1.8 %)
FLT3 receptor gene mutation, *N (%)*	
- ITD	255 (88.2 %)
- TKD	32 (11.1 %)
- both	2 (0.7 %)
NPM1 N/patients in which mutation was searched (%)	237/289 (82.0 %)
- mutated	146/237 (62.6 %)
- wild-type	91/237 (38.4 %)
IDH1 N/patients in which mutation was searched (%)	27/289 (9.3 %)
- mutated	4/27 (14.8 %)
- wild-type	23/27 (85.2 %)
IDH2 N/patients in which mutation was searched (%)	31/289 (10.7 %)
- mutated	4/31 (12.9 %)
- wild-type	27/31 (87.1 %)
Tp53 N/patients in which mutation was searched (%)	15/289 (5.2 %)
- wild-type	15/15 (100.0 %)
Karyotype analysis at diagnosis, availability *N (%)*	233/289 (80.6 %)
- normal	188 (80.7 %)
- -7	2 (0.9 %)
- del7q	1 (0.4 %)
- -5	1 (0.4 %)
- +8	7 (3.0 %)
- t(v;11)(v;q23)-MLL	1 (0.4 %)
- t(9;11)(p22,q23), MLLT3-MLL	1 (0.4 %)
- t(6;9)(p23,q34), DEK-NUP214	1 (0.4 %)
- complex	7 (3.0 %)
- other	24 (10.3 %)
Risk stratification system , *N (%)*	
▪ Favorable	29/289 (10)
▪ Intermediate	190/289 (65,7)
▪ Adverse	27/289 (9,3)

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ONCORELIEF: A DIGITAL GUARDIAN ANGEL ENHANCING CANCER PATIENT'S WELLBEING AND HEALTH STATUS IMPROVEMENT FOLLOWING TREATMENT

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The burden of cancer is rising globally and is estimated to have reached 18.1 million new cases and 9.6 million cancer deaths in 2018. Despite the rising cancer incidence, improvements in early detection and therapeutic treatment have improved cancer survival. As a consequence, the number of cancer survivors is increasing globally, creating the need to improve not only treatment but also wellness and follow-up care. Cancer treatment often involves combined modalities such as surgery, chemotherapy, and radiotherapy. In the past decades, more effective and targeted therapeutic modalities and less destructive cancer treatments have been developed such as immunotherapy and drug-targeted therapy. Even so, cancer and its treatment have important physical and psychosocial sequelae. ONCORELIEF is a 36-month action that will leverage the above 6 drivers in order to skillfully and methodologically overcome technical challenges, by introducing new approaches that will allow the utilization of big datasets in order to develop a user-centered AI System to facilitate the integration of QoL assessment instruments through the use of PROMs and PREMs in order to improve post-treatment health status, increase the wellbeing, and follow-up care of Acute Myeloid Leukaemia (AML) and Colorectal cancer (CRC).This will be achieved through an intuitive smart digital assistant (Guardian Angel), able to provide personalized support in post-treatment activities and tasks, suggest actions regarding the patients' overall health-status, improved wellbeing and active health-care and ultimately maintain him/her engaged on a wellness journey that will safeguard his/her health over the foreseeable prolonged post-cancer treatment period. To achieve this, ONCORELIEF builds on the combined knowhow of its interdisciplinary industry-driven consortium that brings together state-of-the-art technological skills, design thinking methodology and occupational psychology/health sciences.

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BASELINE CD22 MEAN FLUORESCENT INTENSITY AND INOTUZUMAB OZOGAMICIN FOR RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: We explored the relationship between CD22 fluorescent intensity and outcomes of inotuzumab ozogamicin (InO) treatment in patients treated with InO for relapsed/refractory (R/R) B-cell acute lymhoblastic leukemia at a single center Italian Hematology Institution.

Methods: Adults treated for R/R B at the Bologna Hematology Institute were retrospectively assessed for CD22 fluorescent intensity calculated as the CD22 mean fluorescent intensity (MFI) of the PE channel on blast cells divided by the MFI of the PE channel on CD22- cells within the lymphocyte gate (corresponding to T lymphocytes). Outcomes were analyzed by baseline CD22 MFI.

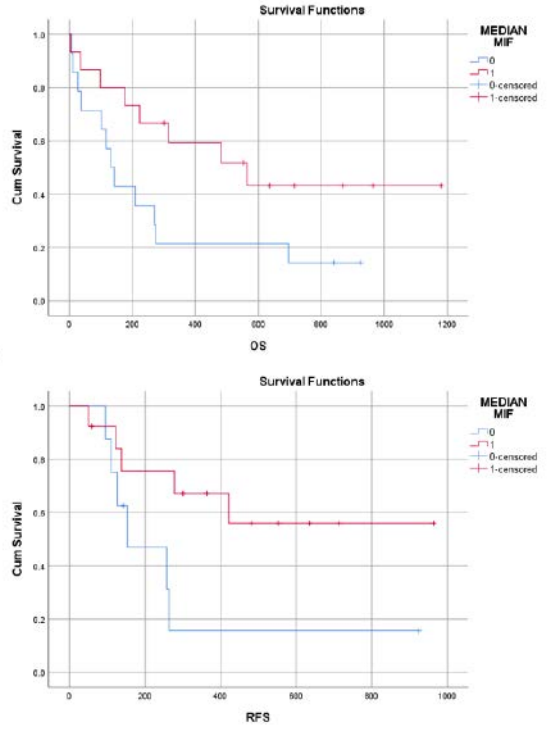

Figure 1.

Results: Of the 33 patients treated from October 2013 to April 2020, 29 (87.9%) were evaluable for CD22 MFI. Median CD22 MFI in the cohort was 112.0 (IQR 52.3 – 242.5). Patients were

divided in wo groups thus defined: patients CD22FIlow below median value and patients CD22FIhigh above median value. Thirty of 33 (90,1%) patients were evaluable for response; ORR was 70% (21/30), 3/30 (10%) patients achieved a PR and 6/30 (20%) were refractory. Of the 21 responsive patients MRD negativity was achieved in 12 of 20 (60%) patients (1 patient not evaluable for MRD status). Twenty-six patients were evaluable for both response and CD22MFI. CR rates in the CD22FIlow and CD22FIhigh were respectively 5/12 (41.7%) and 12/14 (85.7%). Patients with CD22FIlow were less likely to achieve CR after 1st IO cycle (p=0.038). Median OS in the entire cohort was 6,9 months (95% CI 2,7-11,0); OS at 1 year was 40,0% and at 2 years 24.9%. Median PFS was of 8,7 months (95% CI 3,1-14,3); PFS at 1 year was 36,0% and at 2 years 24,7%. When analyzing outcomes by CD22MFI, in the CD22FIlow subgroup OS was 4.4 months (95% CI 2.8-6.0) while CD22FIhigh presented an OS of 18.8 months (95% CI 5.0-32.7) (p 0.052). RFS calculated in responding patients of the two cohorts was in the CD22FIlow 5.1 months (95% CI 2.8-6.0) and in the CD22FIhigh was not reached (p=.06).

Conclusions: In this patient set a CD22FIhigh value is associated with a higher probability to achieve CR. Moreover, patients CD22FIhigh receiving InO treatment seem to have a trend for better OS and PFS than patients with CD22FIlow. A larger sample size analysis is warranted to confirm this preliminary data.

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TREATMENT OPTIONS IN PH-POSITIVE AND PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIAS

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Introduction: Acute lymphoblastic leukemia (ALL) is the uncommon adult hematological malignancy within the structure of morbidity by the hematopoietic neoplasms with primary bone marrow (BM) impairment, being characterized by the involvement of a workable population, prognosis unpredictability and negative socio-economic impact in refractory cases. Around 25% of adults have Philadelphia chromosome-positive (Ph^+) ALL. The accurate diagnosis assists physicians to estimate how leukemia will progress and determine the appropriate treatment.

Methods: The current analytical and descriptive study included two clinical cases with Ph+ and Ph- ALL. These patients were treated and followed up at the Institute of Oncology from Moldova during 2013-2021. Both patients were females of 43 and 31 years old. The diagnosis was asserted by cytochemical staining, immunophenotyping and real-time quantitative PCR of the BM and peripheral blood (PB). The morphological type of hematological malignancies was identified according to the Revised WHO Classifications of Tumors of Hematopoietic and Lymphoid Tissues. BCR-ABL p210 transcript was revealed in the BM and PB of an older patient.

Results: The patients had the ECOG/WHO performance status (PS) 3 at diagnosis. The older patient had 3 years history of the cured left T1N0M0 solid breast cancer before the diagnosis of Ph^+ ALL. The stable complete remission was obtained after the surgical treatment and radiotherapy followed by 4 cycles of the adjuvant chemotherapy. She presented at our hematology department with the spleen and liver enlargement, elevated WBC up to 75000 per mcL, blast cell count of 68% in the PB and 55% in the BM aspirate. The p210 transcript of the BCR-ABL chimeric gene was estimated at 144.65%. The Ph- ALL patient had the spleen and liver enlargement, elevated WBC up to 14000 per mcL, blast cell count of 47% in the PB and 70% in the BM aspirate. L2 subtype of ALL was confirmed in all cases. Both patients proved to be CD20 negative. ALL-BFM 90 regimen was administered as the first-line treatment in all patients. The complete hematological response (CHR) was achieved in the younger Ph- ALL patient within one month of the induction chemotherapy. The ECOG/WHO PS was recovered up to 0. The older Ph^+ ALL patient failed to obtain the CHR and was switched to the second-line AVAMP protocol combined by the targeted chemotherapy with imatinib mesilate at a dosage of 600 mg daily. The ECOG/WHO PS was restored up to 1. Nevertheless, the Ph+ ALL patient achieved only the partial hematological response even after the chemotherapy with AVAMP regimen and imatinib mesilate.

Conclusions: In L2 subtype of ALL, the younger age, mild leukocytosis and BCR-ABL p210 transcript negativity were associated with much better treatment outcome. The secondary Ph+ ALL proved to be resistant to the conventional chemotherapy and imatinib mesilate.