AMLGlobalPortal

General AML

ASH 2017 | Molecular markers in the diagnosis and prognosis of AML

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During the 59th American Society of Hematology Annual Meeting Atlanta, GA, USA, the AGP were delighted to attend a fascinating oral session, which was solely dedicated to biology, cytogenetics and molecular markers in diagnosis and prognosis for Acute Myeloid Leukemia (AML).

The session was co-chaired by Professor Aaron D. Goldberg, MD, PhD from the Memorial Sloan Kettering Center and Professor Francine E. Garrett-Bakelman, MD, PhD from the University of Virginia School of Medicine.

Abstract 181

Daniel A. Pollyea from the University of Colorado Denver, Aurora, CO, gave a talk about the therapeutic value of the BCL-2 inhibitor, venetoclax (ven), in combination with azacitidine (aza) for elderly AML patients unfit for induction chemotherapy.

The speaker discussed the results of the phase Ib dose escalation/expansion study (NCT02203773) which combined aza at the standard dose and schedule, with ven daily in older AML patients who are not candidates for induction chemotherapy.

In total, 33 AML patients (median age = 75 years) were enrolled and received either 400 mg (n = 23), 800 mg (n = 9) or 1200 mg (n = 1) ven combined with a standard dose of aza. Two patients died of AML before the 7th day of the study.

Key Highlights:

- Median follow up; 351 days (95% CI, 146 – 468)
- Median time to response: 35 days (25-62)
- Overall Response Rate (ORR) in all patients; 91% (30/33)
    - Complete Response (CR) rate; 61% (20/33)
    - CR with incomplete count recovery (CRi); 24% (8/33)
    - Partial Response (PR); 3% (1/33)
    - Three non-responders: one did not achieve IWG response after 2 cycles but had significant blast reduction; two discontinued before one week of therapy for reasons unrelated to toxicity or progression
- Median PFS and OS were not reached with a median follow-up time of nearly one year
- Minimal Residual Disease (MRD) in patients with amenable mutations (n = 18) were measured with digital droplet PCR:
    - Fourteen patients completed more than one cycle of therapy with 3 months of follow-up

- Five patients achieved MRD negative status
- One patient remained on therapy
- Two patients discontinued therapy, for personal reasons and cytopenias (they remain in MRD negative CR and CRi after 24 and 16 months)
- One patients achieved CR and left trial after 3 cycles for personal reasons (continues to be in CR with borderline MRD detectability)

- Ven and aza resulted in rapid eradication of blasts and Leukemic Stem Cells (LSCs) in two patients who achieved MRD negativity by effectively inhibiting oxidative phosphorylation through the electron transport chain complex II in LSCs:

Peripheral Blood Blasts (%)			
	Pre-Treatment	24 Hous Post-Treatment	72 Hours Post-Treatment
Patient 1	71%	50%	16%
Patient 2	81%	72%	34%

The speaker concluded that ven in combination with aza could be an effective and relatively safe alternative for untreated elderly AML patients who are not candidates for induction chemotherapy. He further added that this regimen can effectively target the leukemia stem cell population based on the depth and durability of responses. “LSCs can be specifically targeted by exploiting their metabolic vulnerabilities, this can translate into deep and durable remissions irrespective of traditional adverse risk factors for AML patients”.

Abstract 183

Silke Kapp-Schwoerer from the University Hospital of Ulm, Ulm, Germany, presented results from a study which evaluated the clinical relevance of Nucleophosmin 1 mutated (*NPM1*^{mut}) based Minimal Residual Disease (MRD) monitoring in patients with AML at high risk of relapse.

Bone Marrow (BM) and Peripheral Blood (PB) samples at diagnosis (BM [n = 3,527], PB [n = 2,812], after each treatment cycle (BM [n = 1,790], PB [n = 1,264]) and during follow-up (BM [n = 1,205], PB [1,163]) from 611 AML patients (age 18 to 60 years) who were enrolled in four German-Austrian AML Study group (AMLSG) trials (NCT00146120, NCT00151242, NCT00893399, NCT01477606) were analysed in this study. Patients were treated with idarubicin, cytarabine and etoposide (ICE) as double induction +/- ATRA or GO, or one induction cycle with daunorubicin and cytarabine followed by one to four cycles of high dose cytarabine (n = 363, 59%), or autologous (n = 19, 3%) or allogenic (n = 162, 27%) Hematopoetic Stem Cell Transplantation (HSCT). Sixty-seven patients did not complete/receive consolidation. Median follow-up was 3.2 years. *NPM1*^{mut} transcript ratio (*NPM1*^{mut}/*ABL1* transcripts x 10^4) were determined using cDNA based RQ-PCR.

Key Highlights:

- At diagnosis, *NPM1*^{mut} transcript levels as log_{10} transformed continuous variable did not impact relapse free survival, event free survival, overall survival and cumulative incidence of relapse
- After two cycles of treatment, a > 2 log reduction of *NPM1*^{mut} transcript levels in BM and PB was associated with:
  - Superior OS; BM (P < 0.001), PB *(*P < 0.001)
  - Lower Cumulative Incidence of Relapse (CIR); BM (P = 0.0001), PB (P = 0.001)
- 63/395 (16%) patients became RQ-PCR negative (RQ-PCR^{neg}) in the BM, after two cycles of treatment
  - 4-year CIR in RQ-PCR^{neg} and RQ-PCR^{pos} patients; 10% *vs* 40%, P < 0.0001
  - 4-year OS in RQ-PCR^{neg} and RQ-PCR^{pos} patients; 82% *vs* 63%, P = 0.01
- 129/293 (43%) patients became RQ-PCR^{neg} in the PB after two cycles of treatment which associated with lower CIR but not with a difference in OS
  - 4-year CIR in RQ-PCR^{neg} and RQ-PCR^{poz} patients; 17% *vs* 49%, P < 0.0001
  - 4-year OS was not significantly different; P = 0.07
- At the end of treatment, 129/184 patients were RQ-PCR^{neg} in the BM
  - End-of-treatment CIR in RQ-PCR^{neg} and RQ-PCR^{pos} patients; 22% *vs* 48%, P < 0.0001
  - End-of-treatment OS in RQ-PCR^{neg} and RQ-PCR^{pos} patients; 73% *vs* 61%, P = 0.01
- Significant prognostic impact of RQ-PCR positivity were found in BM after two treatment cycles:
  - In NPM1^{mut} RQ-PCR^{pos} patients
    - Relapse: HR = 4.55, (95% CI, 1.82–11.33), P = 001
    - Death: HR = 2.23, (95% CI, 1.13–4.43), P = 0.020
  - In FLT3-ITD^{pos} patients
    - Relapse: HR = 2.58, (95% CI, 1.21–5.50), P = 0.013
    - Death: HR = 4.83, (95% CI, 2.26–10.32), P < 0.001
  - In DNMT3A^{mut} patients
    - Relapse: HR = 2.51, (95% CI, 1.47–4.28), P < 0.001
    - Death: HR = 2.25, (95% CI, 1.14–4.41), P = 0.017

In summary, *NPM1*^{mut} MRD assessment in the BM after two treatment cycles was highly informative and identified patients at an increased risk of relapse. Additionally, the study revealed that RQ-PCR^{neg} in the BM and PB is an independent factor for better OS and longer remission duration. After two treatment cycles, achievement of RQ-PCR negativity in both, the BM and the PB samples, was significantly associated with a lower CIR, independent of concurrent *FLT3-ITD* and *DNMT3A* mutation status while superior OS was only seen for the BM samples. The *FLT3-ITD/DNMT3A*^{mut} genotype impacted on reduction of *NPM1*^{mut} transcript levels and achievement of RQ-PCR negativity. Furthermore, during follow-up, transcript levels above > 200 were highly predictive for relapse in both BM and PB samples.

Abstract 185

The next talk during this session was given by Patrick Williams, from the University of Texas MD Anderson Cancer Center, Houston, TX, USA. The speaker presented results from a study which investigated how AML suppresses immune responses by expressing cytokines and immune checkpoint ligands as well as drafting suppressive immune cells like regulatory T cells (Tregs).

BM aspirates from 107 patients with newly diagnosed (n = 39) or relapsed (n = 68) AML were analysed by 17 color flow-cytometry assay. The authors evaluated the expression of inhibitory (PD1, CTLA4, LAG3, TIM3) and stimulatory receptors (GITR, OX40, 41BB, ICOS) on T cell subsets: CD4 T effector cells (Teff): $CD3^+CD4^+CD127^{lo/+}Foxp3^-$, CD4 T regulatory cells (Treg): $CD3^+CD4^+CD127^-Foxp3^+$, and CD8 T cells and the expression of their ligands (41BBL, B7-1, B7-2, ICOSL, PDL1, PDL2 and OX40L) on AML blasts. Eight healthy donor BMs were used as controls.

Key Findings:

- BM aspirates from AML patients demonstrated significantly higher frequency of CD45 gated $CD3^+$ total T cell infiltrate compared to healthy donors
- $CD8^+$ T cells expressing PD1 was higher in patients with relapsed and with newly diagnosed AML *vs* healthy donors; 32.8% and 26.4% *vs*5%; $P < 0.01$ and $P = 0.03$ respectively
- CD8+ T-cells expressing OX40 was higher in patients with AML *vs* healthy donors
- $CD8^+$ T cells co-expressing the inhibitory markers PD1 with TIM3 was significantly different in healthy donors *vs* patients with relapsed and newly diagnosed AML
- The frequency of Tregs in BM increased progressively from healthy donors to newly diagnosed AML to relapsed AML, 1.6% *vs*8% ($P < 0.01$) *vs* 4.5% ($P < 0.01$)
- Treg infiltration correlated with $CD8^+$ T cells expressing PD1; 33.6% *vs* 27%, $P = 0.03$
- BM blasts in patients with adverse karyotype more frequently expressed PD-L1 as compared to patients with non-adverse karyotype; 17.5% *vs*8%, $P = 0.03$

The speaker concluded, that the increased Treg and $PD1^+$ CD8 T cell infiltration and the increased PD1+TIM3+ and PD1+LAG3+ CD8 T cells in the BM of patients with AML “indicates immune suppression at multiple levels”. He further added that the “dual contributions by T cells and blasts to immune suppression” suggests that these pathways may play a crucial role in AML patient survival and thus may benefit from immune checkpoint therapy.

Abstract 186

The final talk of the session was presented by Ann-Kathrin Eisfeld, from the Ohio State University, Division of Hematology, Columbus, OH, USA.

During this talk, the speaker presented results from a study which aimed to assess the frequency, clinical and molecular associations and the possible prognostic impact of the Neurofibromin 1 gene (*NF1)* mutations in AML.

In this study, targeted mutation assessment was performed in 1,021 adult patients with de novo AML aged either < 60 years (n = 690) or ≥ 60 years (n = 331) and were treated on a Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology protocols. Overall, 81 cancer- and leukemia-associated genes were analysed using costume-designed targeted next-generation sequencing panels (Miseq), moreover, bi-allelic CEBPA mutation status was assessed by Sanger

sequencing resulting in a total of 82 analysed genes. The mutated genes were assigned to nine previously described functional groups defined based on the genes' biologic functions as follows: NPM1, methylation-related, splieceosome, kinases, transcription factors, RAS, chromatin remodelling, cohesion complex, tumor suppressors.

Key Highlights:

- Fifty-nine *NF1* mutations in 52 patients were found, for an overall frequency of 5.1%
- *NF1* mutations were found throughout the gene, and comprised missense (n = 29), frameshift (n = 21) and nonsense (n = 7) mutations
- *NF1*-mutated patients were more often in the adverse ELN risk category than the *NF1* wild-type patients; 42% *vs* 26%, *P* = 0.02
- Complete Remission (CR) rate in *NF1* wild-type (n = 124) or *NF1* mutated (n = 10) younger patients in the adverse ELN risk group; 53% *vs* 20%, *P* = 0.05
- Overall Survival (OS) in younger *NF1* mutated or *NF1* wild type patients in the adverse ELN risk group; 0.4 *vs*0 year, *P* < 0.001
- In older patients in the ELN adverse risk group, there were no significant outcome differences between patients with and without *NF1* mutations
- Associations among different mutation types (missense, nonsense, frameshift mutations, Thr676 hotspot mutation) and patient outcome were identified:
    - CR rates and survival of patients with missense mutations (n = 16), or other frameshift or nonsense mutations (n = 8) did not differ from those of patients with wild-type *NF1*
    - CR rate in *NF1* Thr676-mutated patients (n = 10) *vs* wild-type/other *NF1* mutations, 50% *vs* 79% (*P* = 0.04)
    - Median OS in *NF1* Thr676-mutated patients *vs* wild-type/other *NF1* mutations, 0.8 *vs*2 years (*P* = 0.01)

In summary, these findings established that *NF1* belongs to the 20 most frequently mutated genes in adult AML, occurring in 5.1% of patients. Additionally, the findings of this study suggests that *NF1* mutation is associated with adverse prognostic outcomes in AML patients treated with standard chemotherapy. *NF1*-mutated patients aged ≥60 have lower CR rates compared to *NF1* wild-type patients.

References

1. Pollyea D.A. *et al.* Venetoclax (Ven) with azacitidine (Aza) for untreated elderly Acute Myeloid Leukemia (AML) patients (Pts) unfit for induction chemotherapy: single center clinical experience and mechanistic insights from correlative studies. Abstract 181. 59th American Society of Hematology Annual Meeting. 2017 Dec 9-12; Atlanta, GA, USA.
2. Kapp-Schwoerer S. *et al.* Clinical relevance of Minimal Residual Disease monitoring in NPM1 mutated AML: a study of the AML Study Group (AMLSG). Abstract 183. 59th American Society of Hematology Annual Meeting. 2017 Dec 9-12; Atlanta, GA, USA.
3. Williams P. *et al.* Checkpoint expression by Acute Myeloid Leukemia (AML) and the immune microenvironment suppresses adaptive immunity. Abstract 185. 59th American Society of Hematology Annual Meeting. 2017 Dec 9-12; Atlanta, GA, USA.
4. Eisfield A.K. *et al.* Neurofibromin 1 gene mutations are recurrent events in adult patients with Acute Myeloid Leukemia (AML), associate with poor outcome and refine the adverse European Leukemianet (ELN) risk category. Abstract 186.

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