Supplementary subject and methods

Patients

Samples were collected from 3 Institutions: the Department of Experimental, Diagnostic and the Specialty Medicine of University of Bologna (Italy, n=121) in Italy, the Research Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences (n=161, of which 98 were previously published\textsuperscript{1,2}, genomic data not released) for samples from Austria, Czech Republic, and Serbia, and the University of Michigan (US, n=114, all previously published\textsuperscript{3}, GSE23452). Samples with acute promyelocytic leukemia were excluded.

REDCap was hosted at Istituto Seràgnoli (Department of Experimental Diagnostic and Specialty Medicine, University of Bologna, Italy).

Cells and DNA isolation

Pre-treatment bone marrow and/or peripheral blood cells were processed by Ficoll-Hypaque. DNA was extracted using AllPrep DNA/RNA MiniKit (QIAGEN) in accordance with manufacturer’s instructions. DNA quality and quantity were assessed using the NanoDrop Spectrophotometer (NanoDrop Technologies).

Samples from CeMM and University of Michigan were processed as previously described\textsuperscript{1–3}.

SNP Array protocol

DNA from AML samples was processed through the following steps: digestion, ligation, amplification and purification, fragmentation, labeling, hybridization, washing, staining and finally scanned to obtain the CEL files, according to manufacturer’s instruction. The fragmentation protocol of the group of samples performed at University of Bologna has been adjusted by increasing the volume of 10x Fragmentation Buffer and Fragmentation Reagent in order to improve the efficiency of DNA fragmented. We used an adjusted fragmentation protocol, with a bigger volume of 10x Fragmentation Buffer and Fragmentation Reagent in order to obtain a better quality of DNA fragmented, under Affymetrix Technical Support’s suggestion.

Detection of chromothripsis

Chromothripsis occurred in 1 or 2 chromosomes per patient characterized by a cluster of breakpoints, regularity of oscillating CN states (2-3 CN states, e.g. from heterozygous deletions to amplifications or from heterozygous deletions to amplifications in more than one copy or to homozygous deletions) within 10 subsequent rearrangements interspersed in diploid regions and a high and variable number of breakpoints.

Chromothripsis events were detected as described in methods, then confirmed by visual inspection using both Nexus CN Software v. 8.0 (BioDiscovery) and R package ”Rawcopy” 36, in order to verify the presence of a CN-LOH in the B-allele frequency (BAF), to assess technical quality (hybridization level and quality of the physical array) and to obtain an extensive overview of chromosomal aberrations.

SNP microarray analysis
The threshold of CN gain and loss were set at 0.15 and -0.15, respectively. Copy Neutral LOH or UPD (Uniparental Disomy) was defined as a region displaying LOH without a CN loss. Chromosomic CNA of at least 1 kb and with a minimum of 8 probes per segment were considered and sex chromosomes were excluded from genomic analysis for the lack of paired normal controls for all cases.

**Microarray statistical analyses**

CEL file reports were exported from Nexus CN (BioDiscovery) v. 8.0 and went through statistical analysis using R v3.3.2 and Bioconductor v3.4 (BioInstaller 1.24.0) with following packages: "org.Hs.eg.db" v3.4.0, "reactome.db" v1.58.0, "clusterProfiler" v3.2.11, "ReactomePA" v1.18.1. All p-values were adjusted for multiple testing with Benjamini-Hochberg method. Fisher's exact test was used to compare frequencies in genes' event between two groups. Genes which are not reported in Atlas of Genetics and Cytogenetics in Oncology and Haematology and which did not contain at least one event at single gene's level with an adjusted p-value lower than $10^{-4}$ in the Fisher exact test comparison were filtered out. For testing at a pathway level, genes were annotated in the Reactome database. Firstly, pathway enrichment analysis was performed at patient level by means of an over-representation test (based on hypergeometric distribution). Then, the adjusted p-values obtained for a certain pathway across all patients were used as predictor variable in a logistic regression model fitted against the case/control classification as dependent variable (0=c, 1=control, ctrl); p-values from all the performed logistic regression tests were in turn adjusted for multiple testing. The significance level was set at $10^{-4}$ (adj-p < $10^{-4}$; CI 99.9999%).

Non-coding genes (LINC- and –IT genes), or genes considered not detectable by the limits of SNP array because involving RNA transcripts (microRNAs, small nucleolar RNAs, antisense RNAs, small cajal body-specific RNAs) or with uncertain function (LOC genes) and highly recurrent as a CN Variant (olfactory receptors, mucins, keratins, ryanodine receptors, cub and sushi multiple domain proteins, neurexins, contactins) were filtered out from the list of CNA.

**Molecular analyses**

The mutational status of exons 5-9 of TP53, exon 12 of NPM1, exons 13-15 and 20 of FLT3 was determined using specific primers for qualitative PCR. Mutated samples where then confirmed by Sanger sequencing, using the same PCR primers. CEBPA, IDH1, IDH2 and TP53 mutations were directly investigated by PCR and Sanger sequencing. Presence of FLT3-tyrosine kinase domain (TKD) mutations was determined by digestion with specific restriction enzymes (Promega), for the detection of FLT3-internal tandem duplication (ITD), insertions in NPM1 and DNMT3A mutations, a denaturing high performance liquid chromatography screening (D-HPLC; Transgenomic) was done, followed by direct Sequencing of D-HPLC-positive samples. The expression of WT1 was quantified with a Real Time PCR assay (Ipsogen WT1 ProfileQuant Kit) using ABL as control gene (sensitivity $10^{-4}$).

In the group of samples from CEMM and University of Michigan, the mutational status of the same genes described above plus RUNX1, CBL, NRAS were assessed as previously described.
Chromosome Banding Analysis

CBA was performed on bone marrow cells after short-term cultures (24 and/or 48 hours). Briefly, the cells were treated with colchicine and hypotonic solution and the pellet was fixed and washed in methanol/acetic acid (3:1). The cells were then re-suspended in fixative and dropped on slides. Karyotypes were examined after G banding technique and described according to International System for Human Cytogenetic Nomenclature (ISCN 2016). Complex karyotype was defined as three or more chromosomal abnormalities in the same clone.

FISH

To characterize the chromosomes involved in chromothripsis, we used whole chromosome painting probes specific for chromosomes 5 and 12, (Kreatech, LeicaBiosystem,Wetzlar, Germany), LSI MYC Dual Color, Break Apart Rearrangement Probe (Vysis, Abbott Molecular, IL, USA), LSI MLL Dual Color, Break Apart Rearrangement Probe (Vysis) CEP17 Spectrum Green/LSI TP53 Spectrum Orange (Vysis), EVII (MECOM) Tricolor Breakapart Probe (Cytocell, Cambridge, UK). The slides were counterstained with DAPI and analyzed using fluorescent microscopes equipped with FITC/TRITC/AQUA/DAPI filter sets and the Genikon imaging system software (Nikon Instruments, Tokyo, Japan).

Clinical Statistical analysis

Clinical data collection included age at diagnosis, de novo/secondary AML, White Blood Cells (WBC) count at diagnosis, therapy information [induction therapy (collected in macro categories: ‘chemotherapy’ – ‘hypomethylating agents’ – ‘best supportive therapy’ for the high grade of variability, overall and in each institution), response to induction, consolidation courses], Hematopoietic Stem Cell Transplant (HSCT), death or last follow-up date, cytogenetic data at diagnosis, molecular data (mutations of TP53, FLT3, NPM1, IDH1, IDH2, DNMT3A, CEBPA, RUNX1, CBL, nRAS, WT1 expression) at diagnosis.

Logistic regression models were applied to identify putative risk factors. Survival analyses were carried out with Kaplan-Meier method and significance was assessed by Log Rank test. Regression models were built based on Cox Hazard Ratio. Analyses and graphs were obtained with IBM SPSS Statistics.
References for supplementary methods

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Supplementary tables

Table S1: Missing value for every parameter considered

| Parameter                        | Valid | Missing |
|----------------------------------|-------|---------|
| chromothripsis                   | 395   | 0       |
| sex                              | 395   | 0       |
| pathology                        | 395   | 0       |
| secondary                        | 372   | 23      |
| de novo                          | 372   | 23      |
| WBC > 100.000/mm³                | 152   | 243     |
| WBC > 30.000/mm³                 | 152   | 243     |
| karyotype                        | 352   | 43      |
| ELN risk                         | 352   | 43      |
| induction_therapy                | 308   | 87      |
| mylotarg in induction            | 274   | 121     |
| induction courses                | 113   | 282     |
| response to induction            | 289   | 106     |
| allogenic HSCT                   | 283   | 112     |
| TP53 loss                        | 395   | 0       |
| MAPD                             | 395   | 0       |
| TP53 mutation status             | 324   | 71      |
| FLT3 mutation status             | 298   | 97      |
| NPM1 mutation status             | 286   | 109     |
| IDH1 mutation status             | 121   | 274     |
| IDH2 mutation status             | 135   | 260     |
| DNMT3A mutation status           | 38    | 357     |
| CEBPA mutation status            | 106   | 289     |
| RUNX1 mutation status            | 87    | 308     |
| CBL mutation status              | 91    | 304     |
| NRAS mutation status             | 95    | 300     |
Table S2: Differences in baseline characteristics and therapy in the population enriched for *TP53* alteration (loss and/or mutation). (Only patients with available data are included)

| Comparison                                                                 | Number of patients with chromothripsis | Number of patients without chromothripsis | $\chi^2$ | p  |
|---------------------------------------------------------------------------|----------------------------------------|------------------------------------------|---------|----|
| HSCT-received                                                              | 14/18                                  | 23/30                                    |         | ns |
| Treated with anthracycline based chemotherapy at diagnosis                 | 10/19                                  | 23/38                                    |         | ns |
| Response to induction                                                      | 3/10                                   | 7/21                                     |         | ns |
| De novo AML                                                                | 15/20                                  | 26/39                                    |         | ns |
| WBC at diagnosis (*>30,000/mm$^3$*)                                        | 0/12                                   | 10/14                                    | .067    |    |
| *NPM1* mutation                                                            | 0/20                                   | 1/34                                     |         | ns |
| *FLT3* mutation                                                            | 0/20                                   | 3/34                                     |         | ns |
Table S3: best 1% scoring REACTOME pathways enriched per amplifications in one or more copy in chromothripsis patients compared with non-chromothripsis patients (sorted by score)

| Pathway Super Category | Pathway Name                                      | Altered genes/genes in the pathway | Q-VAL     |
|------------------------|--------------------------------------------------|------------------------------------|-----------|
| Metabolism             | Glycosaminoglycan metabolism                     | 109/116                            | 7.98E-06  |
| Cell Cycle             | E2F mediated regulation of DNA replication       | 26/30                              | 2.75E-05  |
| Disease                | Constitutive Signaling by Aberrant PI3K in Cancer | 58/59                              | 2.75E-05  |
| DNA Repair             | DNA Repair                                       | 138/141                            | 2.75E-05  |
| Hemostasis             | Platelet activation, signaling and aggregation    | 182/190                            | 2.75E-05  |
| Hemostasis             | Cell surface interactions at the vascular wall    | 98/101                             | 2.75E-05  |
| Hemostasis             | Tie2 Signaling                                   | 18/18                              | 2.75E-05  |
| Immune System          | Signaling by Interleukins                        | 99/107                             | 2.75E-05  |
| Immune System          | Interleukin-2 signaling                          | 38/41                              | 2.75E-05  |
| Immune System          | Signaling by the B Cell Receptor (BCR) signaling | 184/190                            | 2.75E-05  |
| Immune System          | Fc epsilon receptor (FCERI) signaling            | 162/171                            | 2.75E-05  |
| Immune System          | CD209 (DC-SIGN) signaling                       | 20/21                              | 2.75E-05  |
| Metabolism             | Glycerophospholipid biosynthesis                 | 87/90                              | 2.75E-05  |
| Metabolism             | Phospholipid metabolism                          | 133/139                            | 2.75E-05  |
| Signal Transduction    | SOS-mediated signalling                          | 14/14                              | 2.75E-05  |
| Signal Transduction    | Signaling by EGFR                                | 164/168                            | 2.75E-05  |
| Signal Transduction    | Signalling to ERKs                               | 37/37                              | 2.75E-05  |
| Signal Transduction    | Signaling by FGFR                                | 148/151                            | 2.75E-05  |
| Signal Transduction    | Signaling by ERBB4                               | 141/143                            | 2.75E-05  |
| Signal Transduction    | Signaling by Leptin                              | 21/21                              | 2.75E-05  |
| Signal Transduction    | Signaling by FGFR1                               | 148/151                            | 2.75E-05  |
| Signal Transduction    | Signaling by FGFR2                               | 148/151                            | 2.75E-05  |
| Signal Transduction    | Signaling by FGFR3                               | 148/151                            | 2.75E-05  |
| Signal Transduction    | Signaling by FGFR4                               | 148/151                            | 2.75E-05  |
| Signal Transduction    | RHO GTPases Activate Formins                     | 94/102                             | 2.75E-05  |
| Transmembrane transport of small molecules | Ion channel transport                           | 159/177                            | 2.75E-05  |
Table S4: best 1% scoring REACTOME pathways enriched per heterozygous and homozygous deletions in chromothripsis patients compared with non chromothripsis patients (sorted by score)

| Pathway Super Category | Pathway Name                                                                 | Altered genes/genes in the pathway | Q-VAL   |
|------------------------|------------------------------------------------------------------------------|------------------------------------|---------|
| Immune System          | CTLA4 inhibitory signaling                                                   | 9/11                               | 6.95E-13|
| Immune System          | CLEC7A (Dectin-1) induces NFAT activation                                    | 10/12                              | 6.95E-13|
| Metabolism             | Synthesis of PIPs at the late endosome membrane                              | 9/10                               | 6.95E-13|
| Fanconi Anemia pathway | Fanconi Anemia pathway                                                       | 19/24                              | 1.30E-12|
| Metabolism             | alpha-linolenic (omega3) and linoleic (omega6) acid metabolism               | 8/13                               | 1.30E-12|
| Metabolism             | alpha-linolenic acid (ALA) metabolism                                        | 8/13                               | 1.30E-12|
| Metabolism of proteins | Calnexin/calreticulin cycle                                                  | 10/11                              | 1.42E-12|
| Cell Cycle             | G0 and Early G1                                                              | 19/21                              | 1.47E-12|
| Disease                | Diseases of metabolism                                                       | 30/35                              | 1.47E-12|
| Extracellular matrix organization | Laminin interactions                                                      | 24/30                              | 1.47E-12|
| Immune System          | Growth hormone receptor signaling                                            | 21/23                              | 1.47E-12|
| Metabolism             | Heme biosynthesis                                                            | 07/11                              | 1.47E-12|
| Metabolism             | Synthesis of bile acids and bile salts via 24-hydroxycholesterol            | 09/10                              | 1.47E-12|
| Metabolism of proteins | Synthesis of glycosylphosphatidylinositol (GPI)                             | 13/17                              | 1.47E-12|
| Metabolism of proteins | N-glycan trimming in the ER and Calnexin/Calreticulin cycle                 | 12/13                              | 1.47E-12|
| Signal Transduction    | Pre-NOTCH Transcription and Translation                                      | 14/19                              | 1.47E-12|
| Signal Transduction    | The canonical retinoid cycle in rods (twilight vision)                       | 13/16                              | 1.47E-12|
| Transmembrane transport of small molecules | Metal ion SLC transporters                                                  | 21/25                              | 1.47E-12|
| Transmembrane transport of small molecules | Ion transport by P-type ATPases                                              | 24/39                              | 1.47E-12|
Supplementary Figures

Figure S1: Clinical and biological characteristics in patients with and without chromothripsis

Panel (A) WBC at diagnosis in patients with (1) and without (0) chromothripsis, \( p = .040 \); panel (B) FLT3 mutational status in patients with (1) and without (0) chromothripsis: 1 = wild-type FLT3, 2 = FLT3 ITD mutation; 3 = FLT3 TKD mutation; panel (C) age at diagnosis in patients with (1) and without (0) chromothripsis; panel (D) ELN risk in patients with (1) and without (0) chromothripsis: 1 = LR, 2 = INT-1; 3 = INT-2; 4 = HR.
Figure S2: *TP53* altered status and chromothripsis impact on survival and clinical data

OS in patients with (green line) and without chromothripsis (blue line): panel (A) population enriched for *TP53* alteration, p=ns; panel (B) population enriched for *TP53* loss, p=.049; panel (C) population enriched for *TP53* mutation, p=ns. Panel (D) distribution of WBC at diagnosis in the population enriched for *TP53* alteration with (green bars) or without (blue bars) chromothripsis, median test p=ns; panel (E) distribution of age at diagnosis in the population enriched for *TP53* alteration with (green bars) or without (blue bars) chromothripsis, median test p=ns.
Figure S3: Representation of all chromosomes affected by chromothripsis in our cohort of adult AML patients.

Samples’ IDs are reported on the top of each image representing a chromosome affected by chromothripsis: in some patients chromothripsis occurred in 2 different chromosomes. The figure is plotted with R package "Rawcopy" using R 3.3.2.
