SUPPLEMENTARY APPENDIX

# A phase II study of guadecitabine in higher-risk myelodysplastic syndrome and low blast count acute myeloid leukemia after azacitidine failure

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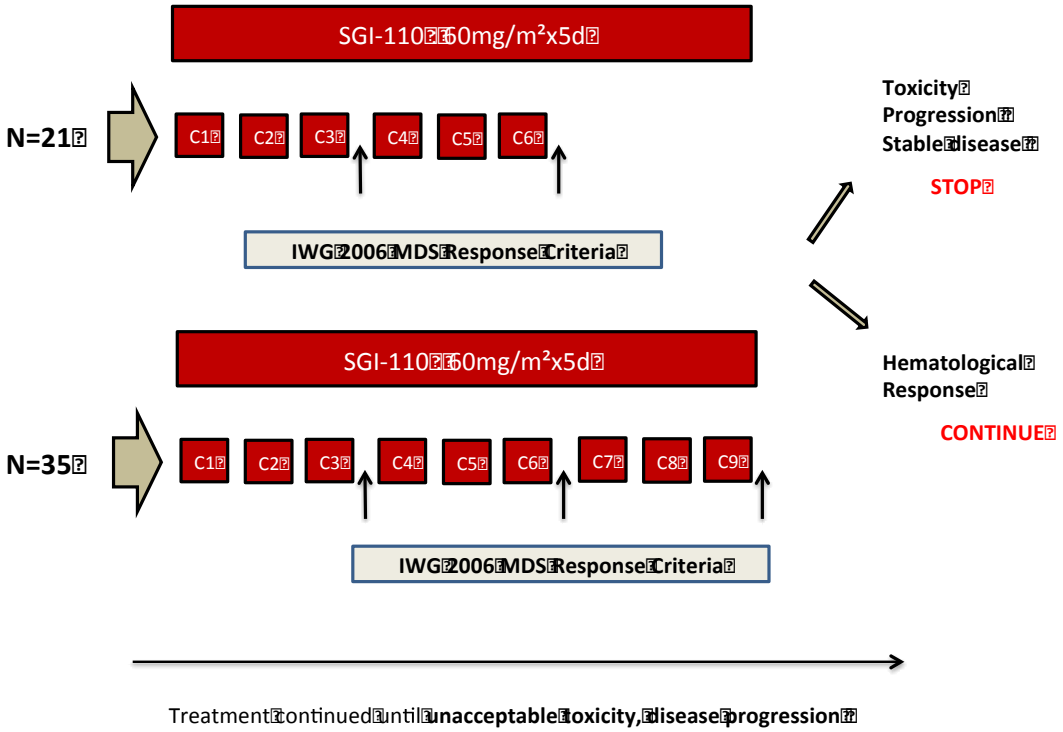
**SUPPLEMENTAL MATERIALS**

## Supplemental methods

## Trial design

**Fig S1:** Study design including the first as well as second patient cohort and different time points for response assessment




## Mutation analysis

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Mutations in a selected panel of 36 genes (**supplementary Table 1**) were screened by a next-generation sequencing (NGS) assay using the Ion AmpliSeq Library Kit 2.0 (384 reactions; Thermo FisherScientific, Carlsbad, CA). Multiplex PCR amplifications were performed from 2 x 5 ng of genomic DNA (pool 1: 284 primer pairs; pool 2: 274 primer pairs). After amplification, barcodes and adaptors were added to amplicons by ligation. Products were subjected to a selective purification on AMPure beads (BeckmanCoulter, Brea,CA). Emulsion polymerase chain reaction (PCR) was performed using the Ion Chef instrument and the Ion 540 Kit-Chef (Thermo Fisher Scientific). Sequencing was performed with the S5XL system (Thermo Fisher Scientific) onto the Ion 540 chip (40 samples per chip). All the samples were also screened for *ASXL1*

(including c.1934dupG; p.G646WfsX12) and *SRSF2* mutations by Sanger sequencing. Base calls were generated by the Torrent Browser software (5.0.4 version) using the included variant caller with an additional plug-in (Thermo Fisher Scientific). The .bam and .vcf files were used for further analysis. The .vcf files were annotated with the Ion reporter software (Thermo Fisher Scientific) and processed for a second analysis of the indexed files using the Sequence Pilot software (4.2.1 version) (JSI Medical Systems, Ettenheim, Germany). Results were compared with selection of variants that will be further considered. For each variant, depth at the variant position (number of mutated reads and unmutated reads) was considered to calculate variant allele frequency, which is the proportion of mutated reads among total reads.

**Methylation analysis**

Global long intersprersed nuclear element (LINE-1) DNA methylation was assessed in 53 patients at baseline and weekly during the first treatment cycle in blood, and on day 28 of cycles 1, 3 and 6 in bone marrow and blood; levels are evaluated through the pyrosequencing technique of 3 representative CpG site in the LINE-1 promoter region in DNA extracted from blood or bone marrow aspirates. All samples were collected in EDTA BD Vacutainer tubes at the participant study sites and shipped immediately at room temperature to Astex Therapeutics Ltd in Cambridge, UK. Bisulfite-conversion and LINE-1 PCR followed by methylation analysis was performed using pyrosequencing technology using a PyroMarkQ24 Sequencer from Qiagen. Relative changes to screening samples for each individual patient were calculated as follows: (Meth Post time point - Meth Screening) / Meth Screening*100.

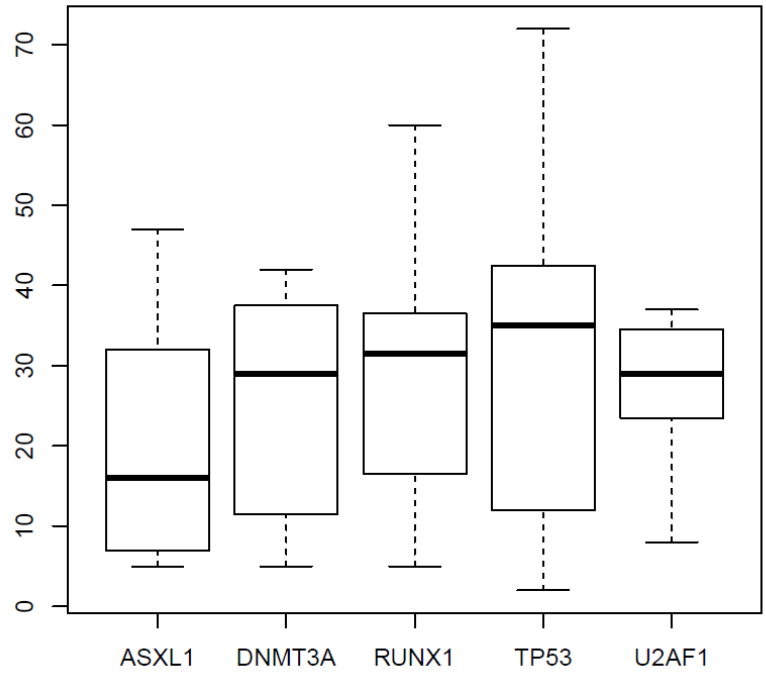
**Table S1**: panel of the 36 genes screened by NGS in all patients included in the study

| Panel of 36 genes | | |
|---|---|---|
| *ASXL1* | *IDH2* | *RUNX1* |
| *BCOR* | *JAK2* | *SETBP1* |
| *BCORL1* | *KIT* | *SF3B1* |
| *CALR* | *KRAS* | *SMC1A* |
| *CBL* | *MPL* | *SMC3* |
| *CSF3R* | *NIPBL* | *SRSF2* |
| *DNMT3A* | *NPM1* | *STAG2* |

| | | |
|---|---|---|
| *ETV6* | *NRAS* | *TET2* |
| *EZH2* | *PHF6* | *TP53* |
| *FLT3-TKD* | *PTPN11* | *U2AF1* |
| *GATA2* | *RAD21* | *WT1* |
| *IDH1* | *RIT1* | *ZRSR2* |

**Supplemental results**

**Figure S2** : VAF of the 5 more frequent somatic mutations found in BM samples of the 56 patients at creening

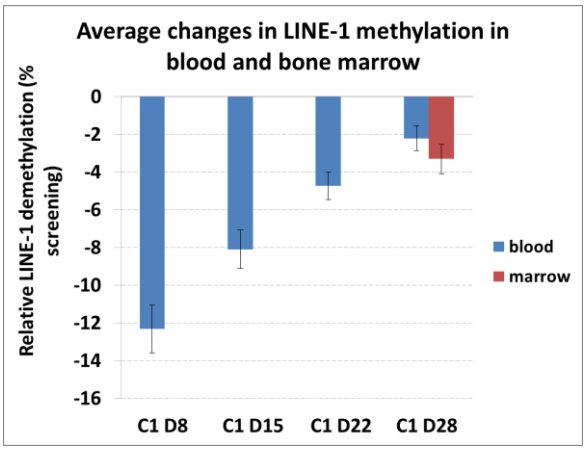



**Methylation:**

The average basal methylation levels of LINE-1 were similar in blood and bone marrow samples with an average of 73% in blood and 71% in bone marrow.

Treatment with Guadecitabine resulted in an average demethylation in blood samples of 12.3% at C1 D8, 8.1% at C1 D15, 4.7% at C1 D22, 2.2% at C1 D28, 2.2% at C3, 1.6% at C6 and 0.1% in blood samples taken at end of study. Bone marrow samples showed demethylation of 3.3% at C1 D28, 2.6% at C3, no change on average at C6 post guadecitabine treatment and demethylation of 0.6% in end of study bone marrow samples.

**Figure S3**: Baseline and average changes in LINE-1 methylation in patient's samples of the study



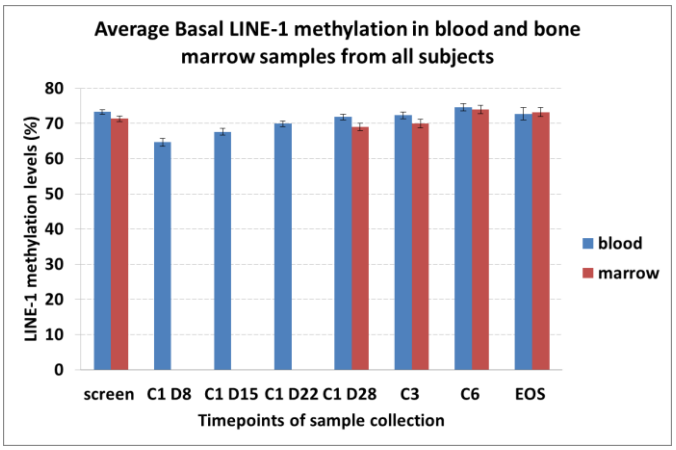




Figure S4 : Overall survival according to demethylation rate