# A randomized phase II trial of azacitidine +/- epoetin-β in lower-risk myelodysplastic syndromes resistant to erythropoietic stimulating agents

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 doi:10.3324/haematol.2015.140988

Received: December 21, 2015.

Accepted: May 19, 2016.

Pre-published: May 26, 2016.

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# Supplemental Data

Supplement to: S Thepot, R Ben Abdelali, et *al*. Randomised phase II trial of Azacitidine +/- EPO in lower risk MDS resistant to ESA

## Supplemental Data Contents

## Supplementary informations:

## Exclusion criteria:

Patients with any of the following: creatinine clearance < 30 ml/min, serum bilirubin > 40 µmol/L, cardiac failure defined by NYHA dyspnea > 2, HIV infection, planned hematopoietic stem cell transplantation, prior treatment with chemotherapy or a hypomethylating agent, were excluded.

## Treatment regimen:

Delayed administration of AZA was allowed in case of predefined hematological toxicity until day 42. AZA dose level reduction was planned in case of persistent cytopenia after day 42, defined in the absence of MDS progression, as > grade 2 non-hematological toxicity, or hematopoietic toxicity leading to hospitalization. Marrow samples were obtained before study entry, after 4, 6 and 18 cycles, and in case of persistent cytopenia, as defined above. Quality of life (QOL), using FACTan and EQ5D questionnaires, was systematically assessed before randomization and after 4, 6 and 18 cycles. Results of QOL studies, currently not available, will not be presented here.

## Conventional cytogenetic and SNP- A karyotyping:

Raw data were analyzed using the Genotyping Console version 4.1 software (Affymetrix). As no germline DNA was available, the following criteria were chosen to define SNP-A abnormality : (i) Copy number aberration (CNA) of more than 1 megabases (Mb) and with less than 50% overlap with germline copy number variants (CNVs) reported in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) (ii) Size and location criteria (telomeric > 8.7 Mb and interstitial, > 25 Mb) were used for identification of somatic UPD as previously described [12]. Subsequently, all CNA and UPD were validated by visual inspection and annotated based on the hg19 human genome assembly.

## Mutational analysis:

Massively parallel sequencing on ABI's SOLiD v4 system (Life Technologies, Carlsbad, CA) or Personal Genome Machine® (PGM™) platform (Life Technologies®) of SureSelect (Agilent, Santa Clara, CA, USA) captured or Ion Ampliseq™ (Life Technologies®) amplified target sequences, respectively. Not previously reported variants were confirmed by direct Sanger sequencing for the following genes: ASXL1*, CBL, DNMT3A, IDH2, RUNX1* and *TET2* using primers described elsewhere [21].

## Sample size justification and statistical analysis:

For continuous variables, values were expressed as median and interquartile range [IQR] and categorical variables were reported as count or percentage and 95% confidence interval (CI95%).

The primary efficacy endpoint was achievement of RBC-TI after 6 cycles, ie major erythroid response (HI-E) response according to IWG 2000 criteria. Such an erythroid response (according to IWG 2000 criteria and IWG 2006) was considered as a binary variable with comparisons across randomization groups based on the exact Fisher test. Missing responses were considered as failures. The search for predictive factors of response was based on multivariate logistic regression. The duration of response was studied in responding patients, computed from the date of response to the date of the first observed event such as relapse, failure, progression, or death. Overall survival was defined as the time between randomization and death. IPSS progression free survival was calculated from the date of randomization to the date of the progression of IPSS. Distributions of the response time, overall survival and IPSS progression free survival were estimated by the Kaplan – Meier method, where patients free of the events of interest were treated as right censored observations at the time of their last follow –up. Differences in these distributions were tested by the log rank test. A Cox model was used for assessing prognostic factors of overall survival. All statistical tests were two-sided, and considered significant for P values $< 0.05$. Analysis was performed using the SAS 9.3 software (SAS Inc, Cary, NC) and R version 3.0.2 (The R Foundation for Statistical Computing)

**Participating centers:**

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**Supplementary Tables**

**Table S1. List of sequenced genes**

| **function** | **Genes** |
|---|---|
| epigenetic modifiers | *TET2, IDH1, IDH2, ASXL1, EZH2, DNMT3A, DNMT3B, KDM6A, SUZ12* |
| splicing | *SF3B1, SRSF2, U2AF1, U2AF2, PRFP40b, ZRSR2, SF1, SF3A1* |
| signaling | *KIT, CBL, BRAF, JAK2, KRAS, NRAS, FLT3, PTPN11, CSF1, CDKN2A, PTEN* |
| Transcription | *RUNX1, GATA1, GATA2, TP53, ETV6, WT1* |

## Table S2. Details of SNP-array karyotyping abnormalities

| Case | Karyotype | nb of lesions | Type of lesion | Chromosome | Cytoband start | Cytoband end | Size (Mb) |
|---|---|---|---|---|---|---|---|
| 1 | normal | 1 | UPD | 14 | q31.1 | q32.33 | 27918 |
| 2 | normal | 1 | loss* | 7 | q22.1 | q22.1 | 2141 |
| 3 | normal | 1 | gain* | 2 | q12.3 | q14.3 | 16291 |
| 4 | normal | 1 | UPD | 4 | q13.3 | q35.2 | 117021 |
| 5 | normal | 1 | loss* | 21 | q22.12 | q22.12 | 1434 |
| 6 | normal | 2 | loss* | 4 | q24 | q24 | 1050 |
| | | | UPD | 13 | q13.2 | q34 | 76250 |
| 7 | normal | 2 | gain* | 12 | all | | 132000 |
| | | | loss* | 13 | q14.13 | q22.1 | 27855 |
| 8 | normal | 3 | UPD | 10 | q25.3 | q26.3 | 16918 |
| | | | UPD | 7 | q21.11 | q36.3 | 73479 |
| | | | UPD | 4 | q22.1 | q35.2 | 101558 |
| 9 | failure | 1 | loss* | 4 | q24 | q24 | 2282 |
| 10 | 47,XY,+8 [20] | 1 | gain | 8 | all | | 146298 |
| 11 | 47,XY,+8 [20] | 1 | gain | 8 | all | | 146298 |
| 12 | 47,XX,+8 [8] / 46,XX [12] | 2 | gain | 8 | all | | 146298 |
| | | | UPD | 2 | p25.3 | p11.2 | 87089 |
| 13 | 47,XX,+8 [14] / 46,XX [8] | 3 | gain* | 19 | p13.3 | p13.3 | 1075 |
| | | | gain | 8 | all | | 146298 |
| | | | UPD | 4 | q22.1 | q35.2 | 100084 |
| 14 | 47,XX,+8 [14] / 46,XX [6] | 1 | gain | 8 | all | | 146298 |
| 15 | 47,XX,+8 [8] / 46,XX [12] | 1 | gain | 8 | all | | 146298 |
| 16 | 47,XY,+8 [11] /45,X,-Y [4] | 1 | gain | 8 | all | | 146298 |
| 17 | 46,X,-Y,+8 [20] | 2 | gain | 8 | all | | 146298 |
| | | | loss | Y | all | | 57773 |
| 18 | 45,X,-Y [13] / 46,idem,+8 [7] | 2 | loss | Y | all | | 57773 |
| | | | gain | 8 | all | | 146298 |
| 19 | 47,XX,del(5q),+8 [20] | 2 | loss | 5 | q22.1 | q33.3 | 45420 |
| | | | gain | 8 | all | | 146298 |
| 20 | 45,X,-Y [24] / 46,XY [1] | 1 | loss | Y | all | | 57773 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | 45,X,-Y [20] | 2 | loss | Y | all | | 57773 |
| | | | UPD | 4 | q21.1 | q35.2 | 77073 |
| 22 | 46,XY,del(13q) [26] / 45,X,-Y [3] / 46,XY [1] | 2 | UPD | 14 | q32.2 | q32.33 | 8798 |
| | | | loss | 13 | q12.3 | q21.1 | 28336 |
| 23 | 46,XY,del(5q) [22] | 1 | loss | 5 | q14.3 | q23.3 | 36774 |
| 24 | 46,XX,del(5q)(q13q32) [25] | 1 | loss | 5 | q14.3 | q33.2 | 70349 |
| 25 | 46,XX,del(5)(q13q33) [6] / 46,idem,del(1)(p34) [8] | 2 | loss | 5 | q14.3 | q34 | 78000 |
| | | | loss | 1 | p36.11 | p35.3 | 20655 |
| 26 | 46,XX,del(13)(q12q21) [9] / 46,XX,del(5)(q31q33) [5] / 46,XX [15] | 3 | loss* | 1 | p36.13 | p36.11 | 10364 |
| | | | loss | 5 | q31.1 | q33.3 | 26396 |
| | | | loss | 13 | q13.1 | q21.1 | 24530 |
| 27 | 46,XY,del(20q) [23] | 1 | loss | 20 | q11.22 | q13.2 | 17438 |
| 28 | 46,XY,del(20q) [18] / 47,idem,+mar [2] | 1 | loss | 20 | q11.22 | q13.31 | 22930 |
| 29 | 46,XY,del(20)(q12) [17] / 46,XY [3] | 1 | loss | 20 | q11.21 | q13.12 | 15100 |
| 30 | 47,XY,add (9)(q22),+9 [8] / 47,idem,del(20)(q12) [10] / 46,XY [2] | 2 | gain | 9 | all p arm | | 66482 |
| | | | loss | 20 | q11.22 | q13.13 | 16567 |
| 31 | 46,XY,del(12)(p11p13) [17] / 46,XY [3] | 2 | UPD | 11 | p15.5 | p13 | 32813 |
| | | | loss | 12 | p13.32 | p12.3 | 14613 |
| 32 | 46,XY,add(3)(p11),t(8;12)(q12-13;p13),del(9)(q13q33),del(17)(q21) [20] | 4 | loss* | 12 | q14.3 | q14.3 | 1302 |
| | | | loss* | 5 | q22.1 | q22.2 | 2181 |
| | | | loss | 3 | p21.31 | p11.1 | 42823 |
| | | | loss | 9 | q21.11 | q33.1 | 48549 |
| 33 | 46,XX,del(11)(q21q22) [16] / 46,XX [4] | 1 | loss | 11 | q21 | q24.1 | 27280 |

* CNA not detected by conventional cytogenetics

**Table S3. Description of gene mutations**

| *Gene* | *Type of mutation* | *Base and amino acid changes* | *Number of mutations* |
|---|---|---|---|
| ***ASXL1*** | frameshift | NM_015338:exon12:c.1900_1922del:p.E635fs | 2 |
| ***ASXL1*** | frameshift | NM_015338:exon12:c.1934dupG:p.G646fs | 2 |
| ***ASXL1*** | frameshift | NM_015338:exon12:c.1919_1929del:p.A640fs | 1 |
| **CBL** | missense | NM_005188:exon9:c.G1259A:p.R420Q | 1 |
| **CBL** | frameshift | NM_005188:exon9:c.1380_1382dup:p.D460dup | 1 |
| ***DNMT3A*** | frameshift | NM_022552:exon8:c.935delC:p.S312fs | 1 |
| ***DNMT3A*** | nonsense | NM_022552:exon9:c.C1066T:p.Q356X | 1 |
| ***DNMT3A*** | frameshift | NM_022552:exon14:c.1571delG:p.C524fs | 1 |
| ***DNMT3A*** | missense | NM_022552:exon14:c.G1628T:p.G543V | 1 |
| ***DNMT3A*** | nonsense | NM_022552:exon17:c.C1966T:p.Q656X | 1 |
| ***DNMT3A*** | frameshift | NM_022552:exon17:c.2043delC:p.I681fs | 1 |
| ***DNMT3A*** | missense | NM_022552:exon20:c.C2374T:p.R792C | 1 |
| ***DNMT3A*** | missense | NM_022552:exon20:c.C2395A:p.P799T | 1 |
| ***DNMT3A*** | frameshift | NM_022552:exon22:c.2532delA:p.K844fs | 1 |
| ***DNMT3A*** | nonsense | NM_022552:exon22:c.C2536T:p.Q846X | 1 |
| ***DNMT3A*** | missense | NM_022552:exon23:c.G2645A:p.R882H | 1 |
| ***DNMT3A*** | missense | NM_022552:exon23:c.G2645C:p.R882P | 1 |
| ***ETV6*** | missense | NM_001987:exon2:c.C145T:p.R49C | 1 |
| ***EZH2*** | missense | NM_001203247:exon7:c.C677A:p.A226D | 1 |
| ***EZH2*** | nonsense | NM_001203247:exon19:c.C2149T:p.Q717X | 1 |
| ***GATA1*** | frameshift | GATA1:NM_002049:exon2:c.31_32insC:p.T11fs | 1 |
| ***IDH2*** | missense | NM_002168:exon4:c.G419A:p.R140Q | 1 |
| ***JAK2*** | missense | NM_004972:exon14:c.G1849T:p.V617F | 4 |

| | | | |
|---|---|---|---|
| ***KDM6A*** | frameshift | NM_021140:exon27:c.3919_3920insG:p.R1307fs | 1 |
| ***KIT*** | missense | NM_001093772:exon8:c.A1256C:p.D419A | 1 |
| ***PRPF40B*** | missense | NM_001031698:exon24:c.C2491G:p.R831G | 1 |
| ***RUNX1*** | frameshift | NM_001001890:exon5:c.885_886insTC:p.S295fs | 1 |
| ***SF3B1*** | missense | NM_012433:exon14:c.G1866C:p.E622D | 8 |
| ***SF3B1*** | missense | NM_012433:exon14:c.A1865T:p.E622V | 1 |
| ***SF3B1*** | missense | NM_012433:exon14:c.C1873T:p.R625C | 4 |
| ***SF3B1*** | missense | NM_012433:exon14:c.C1986A:p.H662Q | 4 |
| ***SF3B1*** | missense | NM_012433:exon14:c.A1997G:p.K666R | 3 |
| ***SF3B1*** | missense | NM_012433:exon14:c.G1998C:p.K666N | 1 |
| ***SF3B1*** | missense | NM_012433:exon15:c.A2098G:p.K700E | 38 |
| ***SF3B1*** | missense | NM_012433:exon16:c.G2225A:p.G742D | 1 |
| ***SF3B1*** | missense | NM_012433:exon16:c.G2233C:p.A745P | 1 |
| ***TET2*** | frameshift | NM_001127208:exon3:c.2290dupG:p.Q764fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon10:c.4317_4318insG:p.K1439fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon11:c.5055delT:p.N1685fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon11:c.5318_5319insC:p.F1773fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon11:c.5324delG:p.S1775fs | 1 |

| *Gene* | *Type of mutation* | *Base and amino acid changes* | *Number of mutations* |
|---|---|---|---|
| ***TET2*** | frameshift | NM_001127208:exon3:c.1143delC:p.F381fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon3:c.1927_1928insCA:p.S643fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon3:c.3202delC:p.Q1068fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon4:c.3412delC:p.Q1138fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon11:c.5225_5229del:p.1742_1743del | 1 |
| ***TET2*** | frameshift | NM_001127208:exon7:c.3945delC:p.D1315fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon3:c.3347_3348insA:p.I1116fs | 1 |
| ***TET2*** | frameshift | NM_001127208:exon7:c.3811_3812insG:p.C1271fs | 1 |
| ***TET2*** | missense | NM_001127208:exon11:c.C5597G:p.P1866R | 1 |
| ***TET2*** | missense | NM_001127208:exon11:c.T5615C:p.L1872P | 1 |
| ***TET2*** | missense | NM_001127208:exon11:c.T5692C:p.S1898P | 1 |
| ***TET2*** | missense | NM_001127208:exon3:c.A1118G:p.Q373R | 1 |
| ***TET2*** | missense | NM_001127208:exon3:c.G3404A:p.C1135Y | 2 |
| ***TET2*** | missense | NM_001127208:exon6:c.C3655T:p.H1219Y | 1 |
| ***TET2*** | missense | NM_001127208:exon7:c.G3866T:p.C1289F | 1 |
| ***TET2*** | missense | NM_001127208:exon8:c.A4034G:p.Y1345C | 1 |
| ***TET2*** | missense | NM_001127208:exon8:c.T3998A:p.M1333K | 1 |
| ***TET2*** | missense | NM_001127208:exon9:c.C4100T:p.P1367L | 1 |
| ***TET2*** | missense | NM_001127208:exon9:c.C4138T:p.H1380Y | 1 |
| ***TET2*** | missense | NM_001127208:exon9:c.G4054A:p.E1352K | 1 |
| ***TET2*** | missense | NM_001127208:exon9:c.G4097A:p.R1366H | 1 |
| ***TET2*** | nonframeshift | NM_001127208:exon6:c.3641_3642insGGA:p.R1214delinsRE | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.148_149insTCT:p.G50delinsVX | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C1630T:p.R544X | 1 |

| | | | |
|---|---|---|---|
| ***TET2*** | nonsense | NM_001127208:exon3:c.C1852T: p.Q618X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C1876T : p.Q626X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C2050T:p.Q684X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C2200T:p.Q734X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C2227T:p.Q743X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.C2428CT:p.Q810X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.G1692A:p.W564X | 1 |
| ***TET2*** | nonsense | NM_001127208:exon3:c.G2995T:p.E999X | 1 |
| ***TET2*** | splice site | NM_001127208:exon6:c.3595-2A>T | 1 |
| ***TP53*** | missense | NM_000546:exon5:c.A478T:p.M160L | 1 |
| ***U2AF1*** | missense | NM_001025203:exon2:c.C101T:p.S34F | 2 |
| ***U2AF1*** | missense | NM_001025203:exon6:c.A470C:p.Q157P | 1 |
| ***ZRSR2*** | frameshift | NM_005089:exon8:c.708delC:p.F236fs | 1 |
| ***ZRSR2*** | frameshift | NM_005089:exon11:c.1116_1117insT:p.D372fs | 1 |
| ***ZRSR2*** | missense | NM_005089:exon8:c.C571T:p.H191Y | 1 |

## Table S4: azacitidine reports that included lower risk MDS

| Report | N (%) of lower risk pts) | Transfusion dependent patients before AZA | ESA failure before AZA | AZA number of days | Transfusion independency after AZA | Hematologic improvement after AZA | Median response duration |
|---|---|---|---|---|---|---|---|
| Musto, 2010 | 74(100%) | 83,8% | 58% | 5d in 39% | NR | 45.9% | 6 months |
| Lyons, 2009 | 151(63%) | 48%<br>43%<br>50% | NR | 5-2-2 d<br>5-2-5 d<br>5d | 44%<br>44%<br>46% | 47%<br>44%<br>50% | 473 d<br>387 d<br>not reached |
| Silverman,2002 | 191(23%) | 63% | NR | 7d | 45% | 47%<br>35%** | 9 months |
| Tobiasson,2014 | 30 (100%) | 100% | 100% | 5 d | 17% | 30% | 5 months |
| Falantes, 2015 | 27 | 96% | NR | 5 d in 37% | NR | 40,7% | |
| Fili, 2013 | 32 | 81% | 69% | 5 d | NR | 41% | 10 months |

NR: not reported

** 5 days schedule after an initial observation period