Prognostic Impact of Minimal Residual Disease in AML

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Prognostic impact of minimal residual disease in AML

• Achievement of complete remission (CR) is the most important prerequisite for cure and long-term survival of patients with acute myeloid leukemia (AML)

• The increasing number of new molecular markers and the development of novel technologies [real-time quantitative polymerase chain reaction (RQ-PCR), multi-color flow cytometry, digital polymerase chain reaction (dPCR), next-generation sequencing (NGS)] allow to measure minimal residual disease (MRD) with high sensitivity

• MRD allows to refine our current definition of morphological CR

• New response category proposed by the 2017 ELN recommendations: “Complete remission without MRD” (CR\textsubscript{MRD-})
Prognostic impact of minimal residual disease in AML

**MRD monitoring: Clinical implications**

- Treatment decision making, in particular within the context of allogeneic stem cell transplantation (alloSCT)
- Early detection of relapse
- Guiding pre-emptive therapy
- Monitoring of treatment effects (novel drugs)
Prognostic impact of minimal residual disease in AML

Detection thresholds of various MRD techniques compared to traditional clinical complete remission

Real-time quantitative polymerase chain reaction (RQ-PCR)

So far, MRD monitoring in AML has been restricted to distinct AML subtypes mainly characterized by gene fusions resulting from translocations/inversions.

Molecular targets for MRD monitoring:
- PML/RARA
- RUNX1/RUNXT1
- CBFB/MYH11
- BCR/ABL
- (KMT2A/MLLT3)
- NPM1

Approximately 50% of all AMLs have one of these molecular targets.

NPM1 mutations (~30%)
Molecular markers not suitable for RQ-PCR based MRD monitoring

- Gene mutations being present in pre-leukemic hematopoietic cells and/or persist during clinical remission:
  - DNMT3A
  - TET2
  - ASXL1
  - IDH1/2

- Gene mutations with heterogeneous breakpoints and/or long PCR products (> 150 bp):
  - FLT3-ITD
  - MLL-PTD
  - CEBPA
  - RUNX1
  - TP53

Can be monitored by NGS
Prognostic impact of minimal residual disease in AML: **Important Issues**

- Most, if not all studies published so far are retrospective and MRD was not included as a primary or secondary endpoint.

- Studies were performed on heterogeneous patient populations with respect to age, treatment, cohort size, or type of material.

- MRD monitoring has not been standardized yet; existence of different MRD assays with distinct sensitivities and definitions for "MRD negativity".

- Studies are not comparable with regard to cut-off values / values for transcript levels / copy numbers.

- In most studies, achievement of MRD-negativity / RQ-PCR-negativity after two cycles of therapy and/or at the end of treatment was significantly associated with outcome.
Prognostic impact of minimal residual disease in AML: Current Data

**Acute Promyelocytic Leukemia**


- Prospective study on 406 newly diagnosed adult APL pts (MRC AML15 trial)
- 6,727 serial BM/PB samples (2,276 paired samples) were analyzed by RQ-PCR
- At the end of treatment achievement of RQ-PCR-negativity was highly predictive for clinical relapse and relapse-free survival (RFS)
- Persistent PCR positivity and molecular relapse were significantly associated with clinical relapse and RFS
- Pre-emptive therapy with arsenic trioxide prevented progression to overt relapse in the majority of the pts
Prognostic impact of minimal residual disease in AML: Current Data

**Core-binding Factor (CBF) Leukemia**

\[ t(8;21)(q22;q22.1); \text{inv}(16)(p13.1q22) \]

- MRD-negativity at end of treatment in PB impacts clinical outcome – French Intergroup
  
  *Willekens et al., Haematologica (2016) [t(8;21), n=94]*

- Transcript level reduction (3-log) before consolidation II influences relapse risk – French Intergroup
  
  *Jourdan et al., Blood (2013) [t(8;21), n=96; inv(16), n=102]*

- Distinct absolute transcript levels and log reduction after induction I and during follow-up correlate with clinically relevant endpoints – UK MRC15
  
  *Yin et al., Blood (2012) [t(8;21), n=163; inv(16), n=115]*
Prognostic Impact of RUNX1/RUNX1T1 MRD-negativity at the end of treatment

Analysis of n=120 RUNX1/RUNX1T1 positive pts of the AMLSG

Overall survival

Event-free survival

Agrawal M et al., ASH meeting 2016, abstract #1207
MRD monitoring in *NPM1* mutated AML

- In 25-35% of AML, particular in CN-AML (45-60%)
- AML with *NPM1*mut/*FLT3*-ITD<sup>-neg</sup> and *NPM1*mut/*FLT3*-ITD<sup>low-ratio</sup> is associated with favorable outcome
- Older patients with *NPM1*-mutated AML benefit from intensive chemotherapy
  
- Mutant *NPM1* is an excellent target for MRD monitoring
  
  - MRD levels assessed by *NPM1* mutation-specific RQ-PCR provide important prognostic information in AML. *Schnittger et al., Blood 2009;114:2220-31;* [n=252]
  - MRD monitoring in *NPM1* mutated AML: a study from the German-Austrian Acute Myeloid Leukemia Study Group. *Krönke et al., JCO 2011;19:2709-2716;* [n=245]
  - The level of residual disease based on mutant *NPM1* is an independent prognostic factor for relapse and survival in AML. *Shayegi et al., Blood 2013;122:83-92;* [n=155]
  - MRD assessed by WT1 and *NPM1* transcript levels identifies distinct outcomes in AML patients and is influenced by gemtuzumab ozogamicin. *Lambert et al., Oncotarget 2014; 5:6280-8;* [n=77]
Assessment of minimal residual disease in standard-risk AML


- Retrospective study on 437 AML pts (pediatric and adults, NCRI AML17 trial)
- 2569 BM/PB (902/1667) samples were analyzed by RQ-PCR after each treatment cycle and during follow-up; sensitivity $10^{-5}$
- MRD positivity in PB after 2 cycles of therapy was significantly associated with inferior OS (24% vs 73%) and higher risk of relapse (82% vs 30%) after 3 years
- In multivariate analysis MRD positivity in PB was significantly associated with death (HR 4.38) and relapse (HR 5.09)
Assessment of minimal residual disease in standard-risk AML

Impact of concurrent FLT3-ITD

Relapse in pts without FLT3-ITD

Relapse in pts with FLT3-ITD

Assessment of minimal residual disease in standard-risk AML

Impact of concurrent $DNMT3A^{\text{mut}}$

Relapse in pts without $DNMT3A^{\text{mut}}$  
Relapse in pts with $DNMT3A^{\text{mut}}$

MRD monitoring in $\textit{NPM1}$ mutated AML: A Study of the German-Austrian AML Study Group (AMLSG)

Patients

- 611 $\textit{NPM1}^{\text{mut}}$ AML patients (age 18 to 60 years) enrolled in one of 4 AMLSG treatment trials [AMLHD98A (NCT00146120) n=46; AMLSG 07-04 (NCT00151242) n=199; AMLSG 09-09 (NCT00893399) n=256; AMLSG 16-10 (NCT01477606) n=110]

Treatment

- Double induction with ICE (idarubicin, cytarabine, etoposide) -/+ ATRA or GO, or 1 induction cycle with daunorubicin and cytarabine followed by 1 to 4 cycles of high-dose cytarabine (n= 363, 59%), or autologous (n=19, 3%) or allogeneic hematopoietic stem cell transplantation (n=162, 27%); 67 (11%) patients did not complete/receive consolidation

- Median follow-up for all patients/trials: 3.2 years
MRD monitoring in $NPM1$ mutated AML: A Study of the German-Austrian AML Study Group (AMLSG)

**Methods**

- cDNA-based RQ-PCR assays for mutation types A, B, C, D, Jt, 4, Qm, Nm and Km; sensitivity of $10^{-5}$ (type 4) to $10^{-6}$ (A, B, C, D, Qm, Nm, Km, Jt) (Gorello et al., Leukemia 2006)

- MRD levels were defined as the normalized value of $NPM1^{\text{mut}}$ transcripts per $ABL1$ transcripts $\times 10^4$ ($NPM1^{\text{mut}}$ transcript levels)

**Material**

<table>
<thead>
<tr>
<th>Time point</th>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>532</td>
<td>358</td>
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<tr>
<td>Therapy</td>
<td>1790</td>
<td>1264</td>
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<tr>
<td>Follow up</td>
<td>1205</td>
<td>1163</td>
</tr>
<tr>
<td>Total</td>
<td>3527</td>
<td>2785</td>
</tr>
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</table>
Prognostic impact of $NPM1^{\text{mut}}$ transcript levels at the time of diagnosis

BM samples $n=532$

- Median $NPM1^{\text{mut}}$ transcript levels varied between $7.03 \times 10^3$ to $1.13 \times 10^9$ ($NPM1^{\text{mut}}/ABL$ copies $\times 10^4$); median $6.47 \times 10^5$

- No correlation with age, sex, WBC, BM blasts, $FLT3$-ITD and $FLT3$-TKD, $DNMT3A$, $IDH1/2$, $NRAS$ mutation status, karyotype and $FLT3$-ITD/$DNMT3A$ genotypes; except of LDH ($P=0.004$)

- $NPM1^{\text{mut}}$ transcript levels as log$_{10}$ transformed continuous variable did not impact RFS, EFS, OS and cumulative incidence of relapse (CIR)
**Prognostic impact of $NPM1^{\text{mut}}$ transcript levels during treatment**

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>Median</th>
<th>Range</th>
<th>Bone Marrow</th>
<th>Pts</th>
<th>Relapse</th>
<th>Death</th>
<th>Peripheral Blood</th>
<th>Pts</th>
<th>Relapse</th>
<th>Death</th>
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<tr>
<td><strong>Median</strong></td>
<td>n</td>
<td>HR p</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>After induction I</td>
<td>1359</td>
<td>0 - 1826000</td>
<td>481</td>
<td>1.45</td>
<td>&lt;0.0001</td>
<td>1.18</td>
<td>0.007</td>
<td>348</td>
<td>1.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>After induction II</td>
<td>45</td>
<td>0 - 904500</td>
<td>381</td>
<td>1.89</td>
<td>&lt;0.0001</td>
<td>1.66</td>
<td>&lt;0.0001</td>
<td>270</td>
<td>1.9</td>
<td>&lt;0.0001</td>
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<tr>
<td>After consolidation I</td>
<td>16</td>
<td>0 - 2183000</td>
<td>342</td>
<td>1.89</td>
<td>&lt;0.0001</td>
<td>1.59</td>
<td>&lt;0.0001</td>
<td>256</td>
<td>1.99</td>
<td>&lt;0.0001</td>
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<tr>
<td>After consolidation II</td>
<td>6</td>
<td>0 - 2875000</td>
<td>256</td>
<td>1.92</td>
<td>&lt;0.0001</td>
<td>1.85</td>
<td>&lt;0.0001</td>
<td>176</td>
<td>2.92</td>
<td>&lt;0.0001</td>
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<tr>
<td>After consolidation III</td>
<td>3</td>
<td>0 - 2368000</td>
<td>209</td>
<td>2.18</td>
<td>&lt;0.0001</td>
<td>1.68</td>
<td>&lt;0.0001</td>
<td>146</td>
<td>2.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>After allogeneic SCT (as consolidation)</td>
<td>0</td>
<td>0 - 2187000</td>
<td>58</td>
<td>2.55</td>
<td>0.0009</td>
<td>1.55</td>
<td>0.0001</td>
<td>48</td>
<td>13.8</td>
<td>0.01</td>
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<tr>
<td>End of treatment (overall)</td>
<td>2</td>
<td>0 - 2368000</td>
<td>290</td>
<td>2.17</td>
<td>&lt;0.0001</td>
<td>1.58</td>
<td>&lt;0.0001</td>
<td>198</td>
<td>2.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>End of treatment (according protocol)</td>
<td>1.6</td>
<td>0 - 2368000</td>
<td>268</td>
<td>2.15</td>
<td>&lt;0.0001</td>
<td>1.59</td>
<td>&lt;0.0001</td>
<td>183</td>
<td>2.44</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**NOTE:** HR for 10-fold increase in $NPM1^{\text{mut}}$ transcript level
Impact of achievement of RQ-PCR negativity in BM and PB after 2 cycles of therapy

After 2 cycles of therapy in patients in CR

BM, n=395

Overall Survival

RQ-PCR negativity, n=63

RQ-PCR positivity, n=332

$P = 0.01$

PB, n=293

Overall Survival

RQ-PCR negativity, n=129

RQ-PCR positivity, n=164

$P = 0.07$

Cumulative Incidence of Relapse

RQ-PCR negativity, n=59

RQ-PCR positivity, n=328

$P < 0.0001$

RQ-PCR negativity, n=125

RQ-PCR positivity, n=162

$P < 0.0001$
### Prognostic impact of $\textit{NPM1}^{\text{mut}}$ transcript levels in BM after 2 cycles of therapy

BM, n=395 in patients in CR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>$P$</td>
<td>HR</td>
<td>95% CI</td>
<td>$P$</td>
<td></td>
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<tr>
<td>$\textit{NPM1}^{\text{mut}}$ cont. variable</td>
<td>1.87</td>
<td>1.58-2.21</td>
<td>$&lt;0.001$</td>
<td>1.44</td>
<td>1.24-1.69</td>
<td>$&lt;0.001$</td>
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</tr>
<tr>
<td>$\textit{FLT3}$-ITD</td>
<td>2.32</td>
<td>1.09-4.95</td>
<td>$0.02$</td>
<td>4.94</td>
<td>2.31-10.55</td>
<td>$&lt;0.001$</td>
<td></td>
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<tr>
<td>$\textit{FLT3}$-TKD</td>
<td>0.721</td>
<td>0.37-1.37</td>
<td>0.32</td>
<td>1.21</td>
<td>0.65-2.25</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.28</td>
<td>0.99-1.65</td>
<td>$0.05$</td>
<td>1.28</td>
<td>0.96-1.71</td>
<td>0.08</td>
<td></td>
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<tr>
<td>BM blasts</td>
<td>1.00</td>
<td>0.99-1.01</td>
<td>0.70</td>
<td>1.00</td>
<td>0.99-1.01</td>
<td>0.27</td>
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<tr>
<td>LDH</td>
<td>1.35</td>
<td>0.59-3.05</td>
<td>0.47</td>
<td>0.94</td>
<td>0.42-2.07</td>
<td>0.88</td>
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<tr>
<td>WBC</td>
<td>0.90</td>
<td>0.61-1.33</td>
<td>0.61</td>
<td>0.89</td>
<td>0.58-1.39</td>
<td>0.63</td>
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</tr>
<tr>
<td>$\textit{DNMT3A}$</td>
<td>2.09</td>
<td>1.21-3.59</td>
<td>$0.007$</td>
<td>1.96</td>
<td>0.99-3.86</td>
<td>$0.05$</td>
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<tr>
<td>Allogeneic SCT</td>
<td>0.84</td>
<td>0.37-1.91</td>
<td>0.68</td>
<td>0.74</td>
<td>0.31-1.74</td>
<td>0.49</td>
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<tr>
<td>$\textit{FLT3}$-ITD/$\textit{DNMT3A}$</td>
<td>0.81</td>
<td>0.33-2.01</td>
<td>0.66</td>
<td>0.68</td>
<td>0.27-1.65</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: HR for 10-fold increase in $\textit{NPM1}^{\text{mut}}$ transcript level
Impact of concurrent FLT3-ITD on clinical outcome

After 2 cycles of therapy in patients in CR
BM, n=395

CIR in pts without FLT3-ITD

CIR in pts with FLT3-ITD

RQ-PCR negativity, n=42
RQ-PCR positivity, n=221

$P < 0.003$

RQ-PCR negativity, n=17
RQ-PCR positivity, n=107

$P < 0.006$
Impact of concurrent $DNMT3A$ mutation on clinical outcome

After 2 cycles of therapy in patients in CR BM, n=395

CIR in pts without $DNMT3A^{\text{mut}}$

\[ P < 0.004 \]

RQ-PCR positivity, n=123

RQ-PCR negativity, n=41

CIR in pts with $DNMT3A^{\text{mut}}$

\[ P < 0.04 \]

RQ-PCR positivity, n=173

RQ-PCR negativity, n=15
Impact of $NPM1^{\text{mut}}$ transcript levels during follow-up period

$NPM1^{\text{mut}}$ transcript level in BM $> 200; \ n=82$

Median time to relapse: 1.7 months after exceeding the cut-off
Impact of concurrent \textit{FLT3-ITD/DNMT3A} mutations on kinetics of $NPM1^{\text{mut}}$ transcript levels

- DNMT3A neg + FLT3 neg
- DNMT3A pos + FLT3 pos

<table>
<thead>
<tr>
<th>Samples, n</th>
<th>140</th>
<th>111</th>
<th>95</th>
<th>70</th>
<th>114</th>
<th>71</th>
<th>98</th>
<th>43</th>
<th>92</th>
<th>19</th>
<th>83</th>
<th>16</th>
<th>1</th>
<th>0</th>
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<tbody>
<tr>
<td>Median</td>
<td>$66054$</td>
<td>$67083$</td>
<td>$102$</td>
<td>$133$</td>
<td>$17$</td>
<td>$108$</td>
<td>$5$</td>
<td>$55$</td>
<td>$1$</td>
<td>$54$</td>
<td>$0$</td>
<td>$36$</td>
<td>$11$</td>
<td>---</td>
</tr>
<tr>
<td>Negative, n</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>30</td>
<td>6</td>
<td>39</td>
<td>10</td>
<td>42</td>
<td>3</td>
<td>43</td>
<td>3</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>Negative %</td>
<td>0.0</td>
<td>0.0</td>
<td>7.4</td>
<td>1.4</td>
<td>26.3</td>
<td>8.5</td>
<td>39.8</td>
<td>23.3</td>
<td>45.7</td>
<td>15.8</td>
<td>51.8</td>
<td>18.8</td>
<td>0.0</td>
<td>---</td>
</tr>
</tbody>
</table>
Summary and Conclusions

• In most of the studies achievement of MRD negativity by RQ-PCR is associated with reduced relapse risk and improved survival.

• In $NPM1^{\text{mut}}$ AML the MRD status after two cycles of therapy is clinically relevant and allows the identification of pts at high risk of relapse.

• During follow-up period, cut-off value $> 200 \, NPM1^{\text{mut}}/ABL \times 10^4$ copies is highly predictive for relapse.

• The $FLT3$-ITD/$DNMT3A$ genotype impacts on reduction of $NPM1^{\text{mut}}$ transcript levels and achievement of RQ-PCR negativity, especially in triple positive patients.

• NGS-based MRD monitoring is not established yet; further development of the techniques is ongoing.

• Standardization/guidelines for MRD monitoring are needed.

• Inclusion of MRD monitoring into clinical trials.
SFB 1074 Experimental Models and Clinical Translation in Leukemia
Achievement of RQ-PCR negativity in \(NPM1^{\text{mut}}\) patients according to \(FLT3\text{-ITD/DNMT3A}\) mutation status in BM

After 2 cycles of therapy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>(NPM1^{\text{mut}}) (FLT3\text{-ITD WT DNMT3A WT})</th>
<th>(NPM1^{\text{mut}}) (FLT3\text{-ITD}^{\text{mut}} DNMT3A^{\text{WT}})</th>
<th>(NPM1^{\text{mut}}) (FLT3\text{-ITD WT DNMT3A}^{\text{mut}})</th>
<th>(NPM1^{\text{mut}}) (FLT3\text{-ITD}^{\text{mut}} DNMT3A^{\text{mut}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>RQ-PCR negative (n)</td>
<td>30 (26%)</td>
<td>14 (25%)</td>
<td>10 (8%)</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>RQ-PCR positive (n)</td>
<td>84 (74%)</td>
<td>41 (75%)</td>
<td>110 (92%)</td>
<td>65 (92%)</td>
</tr>
<tr>
<td>% negative</td>
<td>26%</td>
<td>25%</td>
<td>8%</td>
<td>8%</td>
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</tbody>
</table>

\(P=0.0002\)
Achievement of RQ-PCR negativity in $NPM1^{\text{mut}}$ patients according to $FLT3$-ITD/$DNMT3A$ mutation status in BM

End of treatment

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$NPM1^{\text{mut}}$</th>
<th>$NPM1^{\text{mut}}$</th>
<th>$NPM1^{\text{mut}}$</th>
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<tbody>
<tr>
<td></td>
<td>FLT3-ITD WT</td>
<td>FLT3-ITD WT</td>
<td>FLT3-ITD WT</td>
<td>FLT3-ITD WT</td>
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<tr>
<td></td>
<td>DNMT3A WT</td>
<td>DNMT3A WT</td>
<td>DNMT3A WT</td>
<td>DNMT3A WT</td>
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<tr>
<td>RQ-PCR negative (n)</td>
<td>53 (55%)</td>
<td>21 (60%)</td>
<td>36 (37%)</td>
<td>17 (40%)</td>
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<tr>
<td>RQ-PCR positive (n)</td>
<td>44 (45%)</td>
<td>14 (40%)</td>
<td>61 (63%)</td>
<td>26 (60%)</td>
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<tr>
<td>% negative</td>
<td>55%</td>
<td>60%</td>
<td>37%</td>
<td>40%</td>
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$P=0.02$