Exploratory analysis of 1,936 SNPs in ADME genes for association with busulfan clearance in adult hematopoietic stem cell recipients

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Introduction
- Busulfan is used in preparative regimens prior to hematopoietic stem cell transplantation (HSCT).
- Busulfan has a narrow therapeutic index and exposure is related to the interindividual variability in PK.

Objectives
- To identify biomarkers for busulfan clearance by interrogating 1,936 variants in 225 ADME related genes.

Results

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Exploratory Cohort</th>
<th>Replication Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>Age (mean std.)</td>
<td>53 ± 11</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>Sex</td>
<td>Male = 54 Female = 28</td>
<td>Male = 51 Female = 27</td>
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</tbody>
</table>

- Top SNPs and haplotypes were replicated in an independent cohort of adult patients.

- 33 SNPs and 4 haplotypes explained 64% (adjusted R²) of variance in busulfan clearance (p=0.001).

- These genetic variants, located in GSTA5, CYP2C19, CYP3A42 (2 haplotypes), ABCB4, SLC22A4 and SLC7A8, were replicated in the second cohort.

- The GSTA5 (rs4715354 and rs7746993) haplotype remained significantly associated with busulfan clearance (p=0.025).

Discussion
- This is the first study using an exploratory pharmacogenetic approach in 225 genes involved in ADME to explain the interindividual variability in busulfan clearance.
- We applied an explorative approach, with the advantage of the possibility of discovering new pathways and genetic variants.
- A large number of SNPs were evaluated in the screening cohort, which introduces the potential problem of multiple testing and an increase in the risk of finding false positive relations.
- To minimize this risk, the replication cohort was used to confirm the findings from the exploratory cohort.
- Our results confirm the previously identified association between GSTA4 haplotype and busulfan clearance. Unfortunately this variant can only explain 6.5% of the variability in busulfan clearance.
- No additional genetic markers involved in drug metabolism and transport appear to be associated with busulfan clearance.

Methods
- 62 adult patients receiving busulfan iv prior to their HSCT were included in screening cohort and were genotyped using the Drug Metabolizing Enzymes and Transporters (DMET) array, including 1,936 genetic variants in 225.
- Busulfan serum levels were measured at 2.5 and 4.0 hours after the start of the first infusion and on the day of treatment with a validated high performance liquid chromatography assay.
- Busulfan clearance was estimated with a limited sampling (t=2.5, 4) PK model.
- Associations of busulfan clearance with SNPs and haplotypes were initially tested by linear regression analysis with the polymorphism in the additive model.
- SNPs with a MAF ≥ 10% and candidate markers from the initial univariate analysis were analyzed in the multivariate linear regression analysis.
- Top SNPs and haplotypes were replicated in an independent cohort of adult patients undergoing HSCT (N=85).

References: