Truncation Products of Stromal Cell Derived Factor-1 (CXCL12) Quantified By Mass Spectrometry in Patients with Myelodysplastic Syndrome (MDS) or Acute Myeloid Leukemia (AML) Treated with Rigosertib in a Phase I-II Study

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INTRODUCTION

• CXCL12 ([cysteinyl]collectine-derived factor-1, SDF-1), an 8 kDa peptide chemokine (68 amino acids), ligates chemokine receptor 4 (CXCR4) and activates migration of normal and leukemic stem cells from the bone marrow into the blood.

• Rigosertib (Fig. 1) is a synthetic benzyol styrene sulfone with evidence of activity in certain subsets of patients with MDS and AML (1).

• Previously reported: truncation products of CXCL12 in patients with primary myelofibrosis (~29 ng/mL) and polycythemia vera (~31 ng/mL) (2).

• Objective: To characterize & quantify intact CXCL12 and its protease-induced truncation products (Table 1) in plasma of MDS & AML patients before & after treatment with Rigosertib in Phase I-II dose escalation trials.

METHODS

• PATIENTS: MDS (n=8) or AML (n=12). Rigosertib infused continuously for 3 d (dose: 650-1,700 mg/m²).

• Plasma obtained at 0 and 72 h.

• Samples centrifuged at 300 g for 10 min, diluted with equal volume of water and ultrafiltered (30 kDa exclusion).

• Aliquots analyzed by liquid chromatograph/mass spectrometry (LC/MS).

• Multiply charged molecular ions of CXCL12 were determined by transformation of multiply charged molecular ions of CXCL12.

• Molecular mass of CXCL12 obtained by transformation.

• MS: Positive electrospray ionization (ESI) with selected ion monitoring, m/z 980 for intact CXCL12, m/z 952, 940, 929, and 914 for -2 amino acids (aa, KP removed), -3 aa (KPV removed), -4 aa (KPVS removed), and -5 aa (KPVSL removed) truncation products, respectively (Table 1).

• The presence of truncates was confirmed using standards incubated with various proteases by determining molecular masses using ESI. Quantification: Calibration curves were established using synthetic standards.

RESULTS AND COMMENTS

<table>
<thead>
<tr>
<th>Protease</th>
<th>No. amino acids removed</th>
<th>Amino acids removed</th>
<th>Masses monitored, m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipeptidyl peptidase-CD26</td>
<td>2</td>
<td>KP</td>
<td>952</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>3</td>
<td>KPVS</td>
<td>929</td>
</tr>
<tr>
<td>Matrix metalloproteinase; MMP-9*</td>
<td>4</td>
<td>KPVS</td>
<td>929</td>
</tr>
<tr>
<td>Cathepsin G; CG</td>
<td>5</td>
<td>KPVS</td>
<td>915</td>
</tr>
</tbody>
</table>

Abbreviations: K=lysine; P=proline; V=valine; S=serine; L=leucine.

The same number and type of amino acids were also removed by MMP-2.

| Prostateolytic degradation of CXCL12 may be characteristic of the pathobiology of homing and release from the marrow niche in patients with myeloid malignancies and this process changes in response to treatment.

• Our findings suggest that CXCL12 may be a biomarker for patients with MDS or AML who respond to Rigosertib.

• Further investigation of the potential role of intact CXCL12 and its truncation products in plasma in these diseases is warranted.

REFERENCES/DISCLOSURES

1. Garcia-Manero et al., Lancet Oncology 2016; 17; 496

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