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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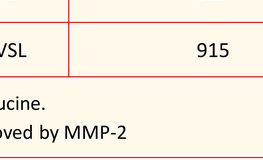
INTRODUCTION

- CXCL12 (stromal cell 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 benzyl styrene sulfone with evidence of activity in certain subsets of patients with MDS and AML (1).
- Previously reported: truncation products of CXL12 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.

STRUCTURE OF RIGOSERTIB

ON 01910Na
Sodium (E)-{N-2-methyloxy- 5(2',4',6'trimethoxy-styrylsulfonyl) Methylenephenyl-amino}acetate



METHODS

- **PATIENTS:** MDS (n=8) or AML (n=12). Rigosertib infused continuously for 3 d (dose: 650-1,700 mg/m^2/d). 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).
- LC: Tosoh C18 column (TSK gel, ODS-100V), gradient elution.
- 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.

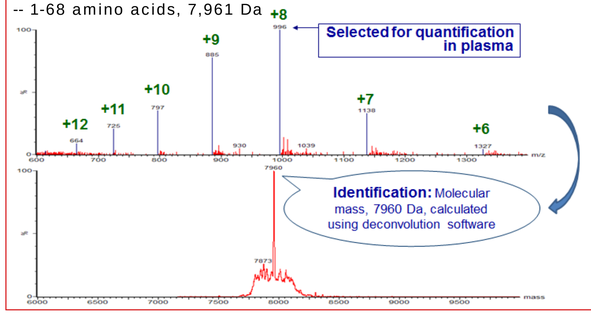
Table 1. Amino acids removed (truncated) from the NH_2 terminal of CXCL12 (1-67 amino acids) by proteolytic enzymes

Protease	No. amino acids removed	Amino acids removed	Masses monitored, m/z
Dipeptidyl peptidase; CD26	2	KP	952
Neutrophil elastase; NE	3	KPV	940
Matrix metalloproteinase; MMP-9*	4	KPVS	929
Cathepsin G; CG	5	KPVSL	915

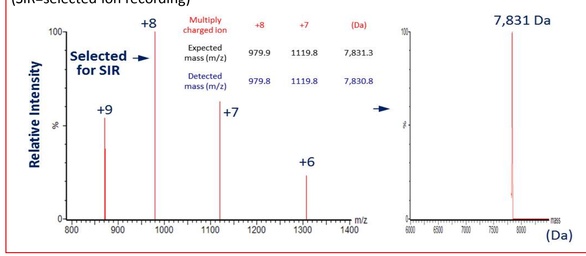
Abbreviations: K=lysine; P=proline; V=valine; S=serine; L=leucine.
*The same number and type of amino acids were also removed by MMP-2

RESULTS AND COMMENTS

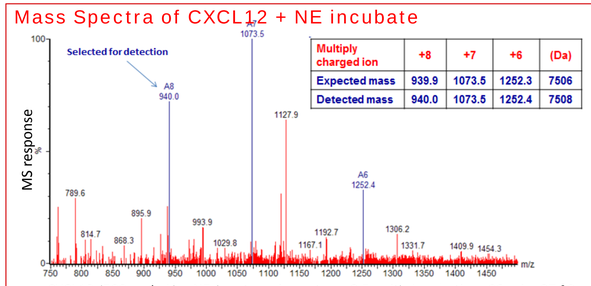
Electrospray (ESI) Mass Spectrum of Recombinant CXCL12
-- 1-68 amino acids, 7,961 Da



Multiply charged molecular ions of CXCL12
Molecular mass of CXCL12 obtained by transformation.
The +8 ion, m/z 980 was used to detect CXCL12 in patients samples
(SIR=selected ion recording)

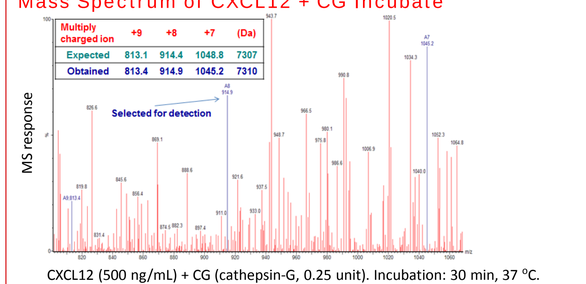


Mass Spectra of CXCL12 + NE incubate



CXCL12 (500 ng/mL) + NE (neutrophil elastase, 0.5 unit). Incubation: 30 min, 37 °C.
Found: truncate, 4-67, 3 aa, KPV removed from SDF-1 sequence.

Mass Spectrum of CXCL12 + CG Incubate



CXCL12 (500 ng/mL) + CG (cathepsin-G, 0.25 unit). Incubation: 30 min, 37 °C.
Found: truncate, 6-67, aa, KPVSL removed from SDF-1 sequence.

Patient Results

PRIOR to Therapy with Rigosertib:

Intact CXCL12: Plasma concentration (ng/mL)	
Normal subjects (n=10)	16.6±9.4
MDS patients (n=8)	3.1± 3.2
AML patients (n=12)	2.4±1.7

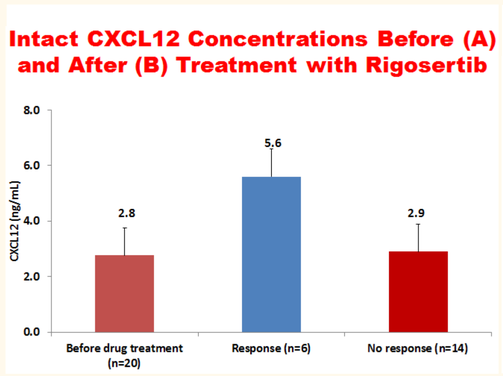
Concentration (ng/mL) of Truncation products

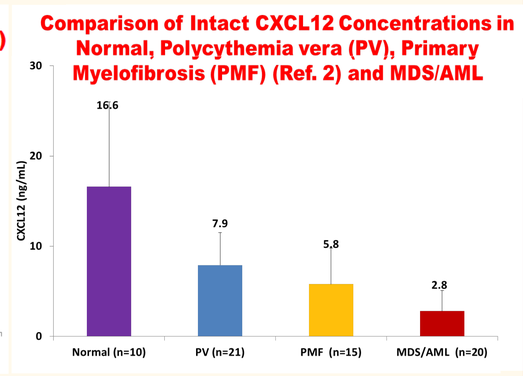
Normal subjects: None detected (≤1.0 ng/mL) --- despite the high concentration of intact CXCL12.
All patients: Truncation products detected/quantified corresponding to the removal of amino acids

	2	3	4	5
MDS	2.5±1.6	2.7±2.1	2.4±1.7	4.2±3.6
AML	3.3± 2.9	2.4±1.7	3.1±2.5	2.2±1.7

AFTER Therapy with Rigosertib:
Partial or complete bone marrow remission: 6/20 patients (International Working Group criteria).

Patients responding to therapy
Intact CXCL12 concentration increased, from 3.4±3.6 ng/mL to 5.6±3.7 ng/mL.
Patients not responding to therapy
Intact CXCL12 concentration: no change, from 2.7±2.2 to 2.9±2.3 ng/mL.





CONCLUSIONS

- Proteolytic 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)
2. Cho, S., Roboz, J. et al. Cancer Res. 70, 3402, 2010).

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