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CXCL12 (SDF-1) and its Truncation Products in Patients with Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML) Receiving ON 01910.Na (Rigosertib) in Phase I Trials

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BACKGROUNDS & OBJECTIVES

- CXCL12 (stromal cell-derived factor, SDF-1), is an 8 kDa peptide chemokine.
- The interaction between CXCL12 and its receptor, CXCR4, plays a pivotal role in the trafficking of hematopoietic stem cells between bone marrow and peripheral blood.
- The CXCL12/CXCR4 axis may play a role in the pathogenesis of myeloid neoplasms. We developed a technique for the determination of intact (full length) CXCL12 and its protease(s)-induced truncation products in plasma from patients with myeloproliferative neoplasms (*Cho et al.*).
- In the present work, we are extending our observations to the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

ON 01910.Na and CXCL12

Fig. 1A: ON 01910.Na

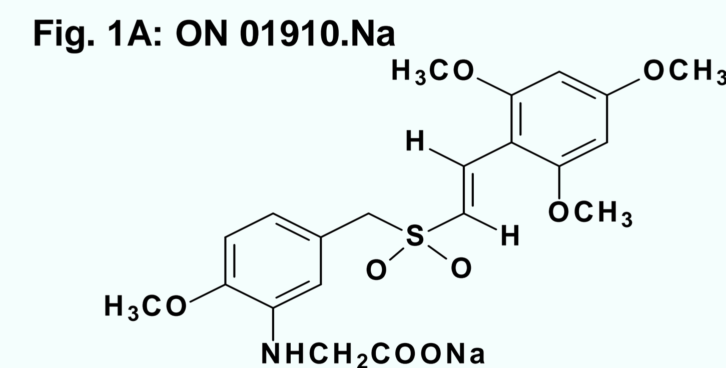


Fig. 1B: CXCL12

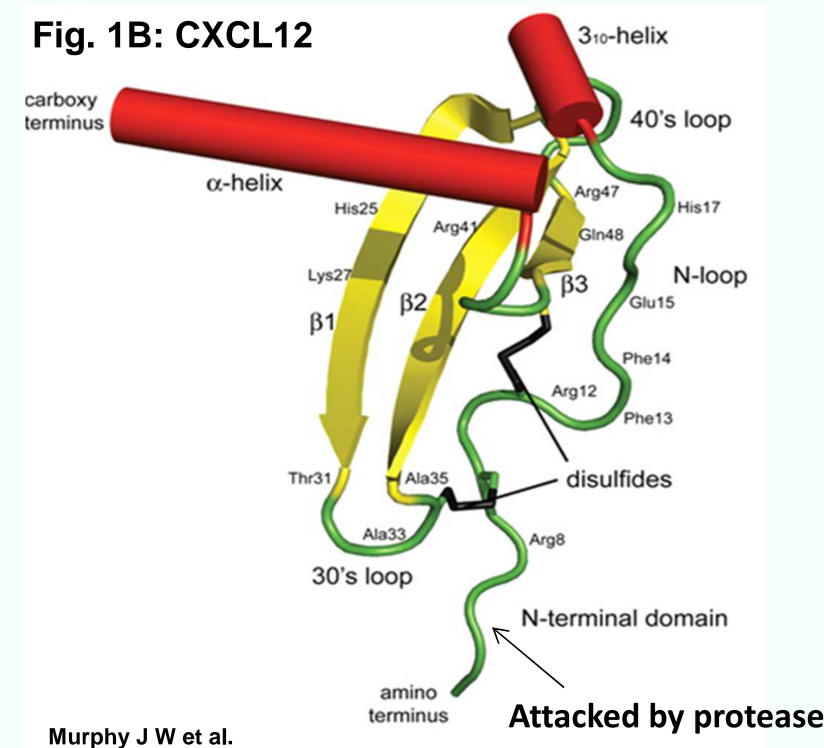


Table. Truncation Products of CXCL12

Protease	# of aa Removed	Removed aa
CD26	2	KP
NE	3	KPV
MMP-2 and 9	4	KPVS
CG	5	KPVSL

- ON 01910.Na (473.5 Da), sodium (E)-{N-[2-methyloxy-5(2',4',6'trimethoxystyrylsulfonyl) methylenephenyl]amino} acetate, is a cell cycle active benzyl styryl sulfone analog with activity against most human cancer cell lines, and against a broad spectrum of human xenografts in mice. The drug is in dose escalation Phase I clinical trials.
- CXCL12/SDF-1 (cell -derived factor-1) is small chemokine belonging to the chemokine family now designated as chemokine (C-X-C motif) ligand 12 (CXCL12).
- The truncated forms of CXCL12 are the product of the action of several serine proteases, including dipeptidyl peptidase-IV, neutrophil elastase, matrix metalloproteinase-2 (MMP-2), MMP-9, and cathepsin G.
- Unlike CXCL12, these truncates either lack the ability to act as a chemoattractant for CD34⁺ cells and/or act as an antagonist to the action of CXCL12.
- CD26: Dipeptidyl peptidase-4; NE: Neutrophil elastase; MMPs: Matrix metalloproteinases; CG: Cathepsin G
- aa: amino acid
- K: lysine, P: proline, V: valine. S: serine, L: Leucine

METHODOLOGY

Obtaining samples from patients

- Patients with MDS or AML (n=15) were treated with ON 01910.Na (rigosertib, 650 – 1,700 mg/m^2), continuous infusion for 3 days.
- Blood samples were taken at 0 and 72 h, in green-top tubes for plasma, followed by centrifugation at 300 *g* for 10 min.
- Plasma samples were diluted with equal volume of water and ultra-filtered using 30 kDa cutoff membranes.

Obtaining mass spectra and quantifying CXCL-12 and its truncated products

- After injecting 10 µL sample aliquots into the electrospray source (positive mode), masses were monitored in 500 – 2,000 Da range (scanning mode);
- Characteristic (diagnostic) multiply charged ions to be used for subsequent analyses were selected from the multiply charged ion profiles;
- Molecular masses of the truncated products were obtained from the multiply charged ion profiles, using a transformation software. Data were confirmed using synthetic standards;
- Selected ion monitoring (SIM) was used to quantify. The following *m/z*-s were monitored for the truncated products: 980 for CXCL-12, and 952 (CD26), 940 (NE), 929 (MMP), and 922 (CG);
- Quantification was accomplished using an CXCL-12 standard (100 ng/mL).

LC/MS Conditions

- Column: Tosoh C18 1.0 mm id, 3.5 cm long, 3 µm particle size; Eluent condition: gradient elution from 20% B to 100% B in 30 min, A) water with 0.1% formic acid, B) acetonitrile with 0.1% formic acid, flow rate: 0.35 mL/min;
- Positive ion electrospray with following conditions - capillary voltage: 3 kV, cone voltage: 15 V. Source temperature: 80 ºC, desolvation temperature: 250 ºC, nitrogen nebulizer gas flow: 80 L/h, desolvation gas flow: 800 L/h.

RESULTS and DISCUSSION

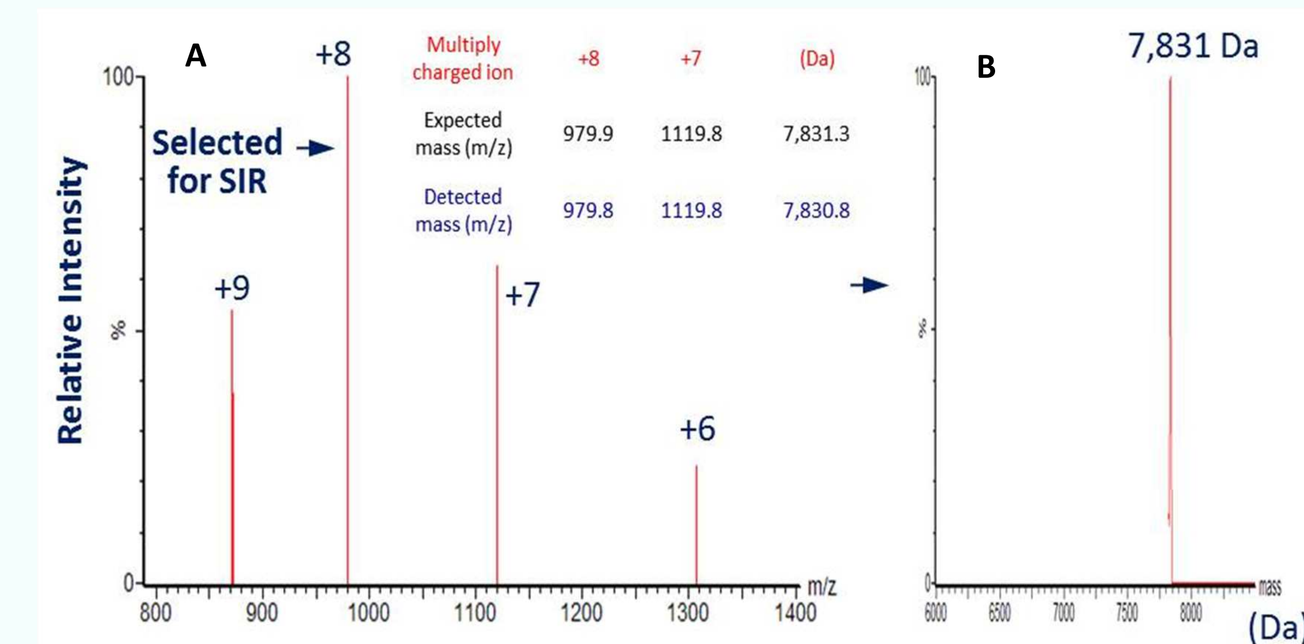
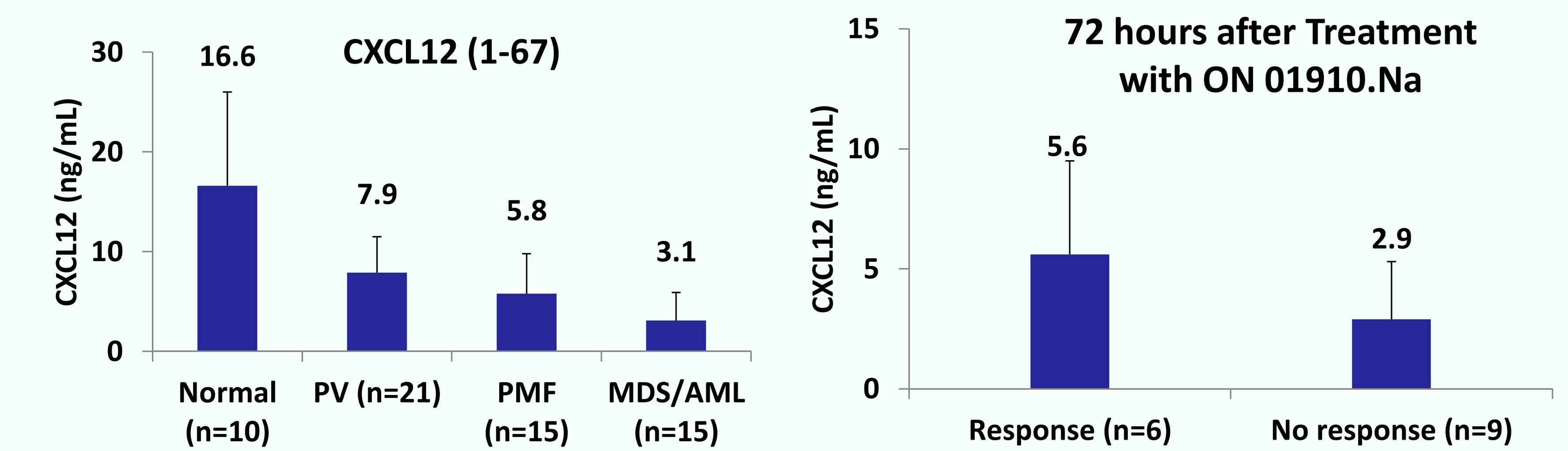


Figure 2. Mass spectrum of CXCL12 (A), Transformed mass spectrum (B)

- In Figure 1A: multiply charged molecular ions of CXCL12. Molecular mass of CXCL12 obtained using MassLynx software for transformation.
- The +8 ion of m/z 980 was used to detect CXCL12 in patients sample.



- Concentration of CXCL12 in normal subjects were 16.6 ± 9.4 ng/mL (n=10).
- In primary myelofibrosis (PMF), the concentrations were 5.8 ± 4.0 ng/mL (n=15), and in polycythemia vera (PV) 7.9 ± 3.6 ng/mL (n=21).
- In MDS/AML, the concentrations were 3.1 ± 2.8 ng/mL (n=15).
- In normal subjects, truncation products due to proteolysis, were not detectable (≤ 1.0 ng/mL).
- The loss of 2, 3, 4, and 5 amino acids (aa) from CXCL12 were confirmed by molecular masses measured using electrospray ionization mass spectrometry. Quantification was completed with synthetic standards.
- The concentrations of the truncation product corresponding to -2 aa (KP), -3 aa (KPV removed), -4 aa (KPVS removed) and -5 aa (KPVSL removed) were 2.7 ± 3.2 ng/mL, 2.4 ± 2.1 ng/mL, 3.8 ± 3.0 ng/mL, and 2.5 ± 2.4 ng/mL, respectively. The total concentration of all truncation products was 11.3 ± 5.8 ng/mL. For comparison, the concentrations of total truncation products was 28.7 ± 19.9 for PMF, and 31.1 ± 7.8 for PV. Patients with these myeloid malignancies have lower CXCL12 concentrations compared to normal controls and high concentrations of proteolytic truncation products which are absent in normal plasma. Of the 15 patients with MDS/AML, treated with continuous infusion of ON 01910.Na (rigosertib), 650 - 1,700 mg/m^2, in a Phase I dose escalation study, 6 patients attained a partial or complete bone marrow remission according to International Working Group criteria. The concentration of intact CXCL12 rose at 72 h in responding patients, whereas those who failed to respond had a little decrease.

CONCLUSIONS

- These data suggest that monitoring intact CXCL12 and its truncation products may provide putative markers of response to treatment with ON 01910.Na, as well as insight into the role of the CXCL12/CXCR4 axis in the pathobiology of these bone marrow diseases.

REFERENCES and ACKNOWLEDGEMENT

- Cho et al. Cancer Res. 2010" 70: 3402-10
- Murphy J W et al. J. Biol. Chem. 2007;282:10018-10027

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