Single-Cell Network Profiling (SCNP) to Evaluate the Proteomic Profiles Associated with ON 01910.Na Treatment of MDS Patients

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ABSTRACT

Background: Single Cell Network Profiling (SCNP) is used to measure simultaneously the effects of multiple modulators (including drugs) on intracellular signaling cascades at the single cell level. ON 01910.Na has been reported to inhibit polo-like kinase 1, PI3-kinase and Akt pathways. In an ongoing phase II study in Int-1, 2 or high risk MDS pts refractory to hypomethylating agents, biomarker assays are being performed to assess CD34+ cell functional signaling profiles associated with biological activity of ON 01910.Na.

Objectives: The objectives were to simultaneously compare the functional effects of a panel of modulators on different signaling pathways (such as the PI3K and the Janus Kinases (Jak) signal transducers and activators of transcription (Stat) pathway) to identify specific proteomic profiles associated with the biological activity of and response to ON 01910.Na in MDS pts.

Methods: MDS patient bone marrow samples were collected at baseline and after treatment cycles 1, 3, 5, & 7. Bone marrow mononuclear cells (BMMCs) were isolated and cryopreserved for longitudinal analysis. Activation of signaling pathways was measured with fluorochrome-conjugated antibodies that recognize p-Erk1/1 (T202/Y204), p-Akt (S473), p-S6 (S235/236), p-Stat1 (Y701), p-Stat3 (S727), and p-Stat5 (Y694). BMMCs were modulated with FMS-like tyrosine kinase 3 ligand (FLTL3), stem cell factor (SCF), granulocyte colony stimulating factor (G-CSF), or granulocyte-monocyte colony stimulating factor (GM-CSF) for 15 minutes. Cells were processed for SCNP by fixation, permeabilization, and incubation with fluorochrome-conjugated antibodies.

Results: SCNP analyses in Pt ON103 (progressed to AML after completion of trial) showed that frequency of CD34+ cells increased during the course of the clinical trial; when modulated with either FLT3L or SCF, compared to baseline findings, CD34+ cells exhibited increased p-S6 and p-Akt responsiveness with treatment; and interestingly, CD34+ cell responsiveness to G-CSF decreased (p-Stat1 and p-Stat5) while no signaling was observed in response to GM-CSF. In contrast, SCNP analyses in Pt ON104 (stable disease), showed that frequency of CD34+ cells was maintained throughout treatment; when modulated with either FLT3L or SCF, CD34+ cells exhibited decreased p-S6, p-Akt, p-Erk (slight); and while CD34+ cell responsiveness to G-CSF decreased (p-Stat1 and p-Stat5), a robust p-Stat5 response was induced by GM-CSF which increased during the course of the clinical trial.

FIGURE 1: SINGLE CELL NETWORK PROFILING (SCNP)

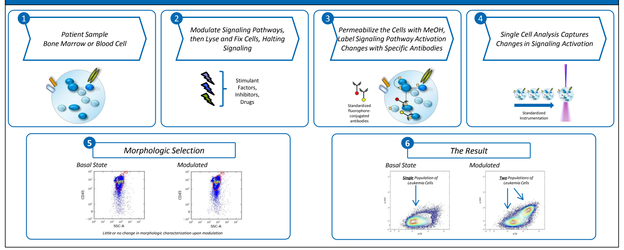
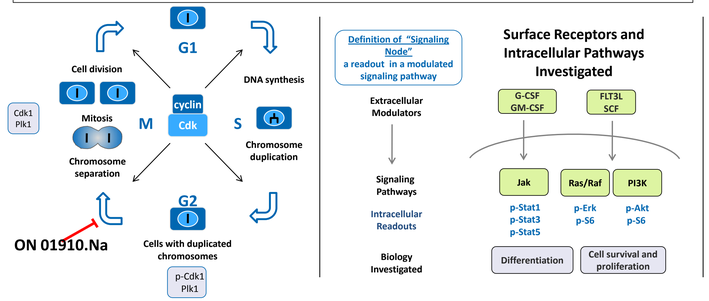


FIGURE 2: ON 01910.NA INHIBITS PLK1 AND THE CELL CYCLE & BIOLOGICAL PATHWAYS ANALYZED IN CD34+ BONE MARROW CELLS

- Dysfunctional apoptosis, proliferation and maturation contribute to MDS pathology.
- The response to external modulation of signaling nodes associated with proliferation / survival and with cellular differentiation were therefore evaluated by SCNP across the time course of treatment with ON 01910.Na.



Patient Eligibility:
- MDS patients with trisomy 8 or classified as Intermediate 1, 2 or high risk
- Not candidates for azacitidine or decitabine, or these therapies failed to induce a response
- Off other therapy for ≥4 weeks

Cytogenetic information for patients analyzed:
- Pt ON103: normal
- Pt ON104: 47,XY,+19[4]/48,+19,+8[16]

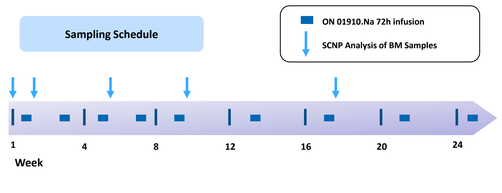


FIGURE 3: GATING HIERARCHY

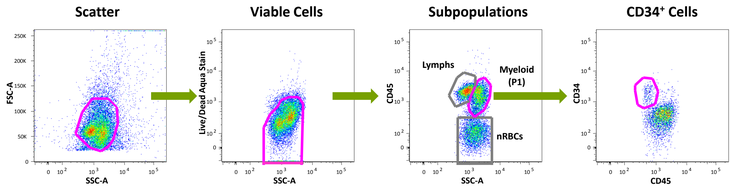


FIGURE 4: CD34 CELL COUNTS INCREASED WITH DISEASE PROGRESSION

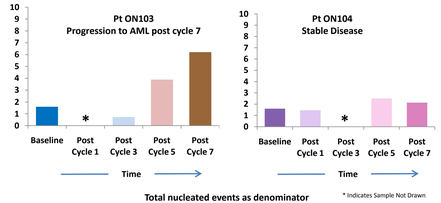


FIGURE 5: CD34+ PROLIFERATION / SURVIVAL SIGNALING RESPONSE TRENDED WITH DISEASE PROGRESSION

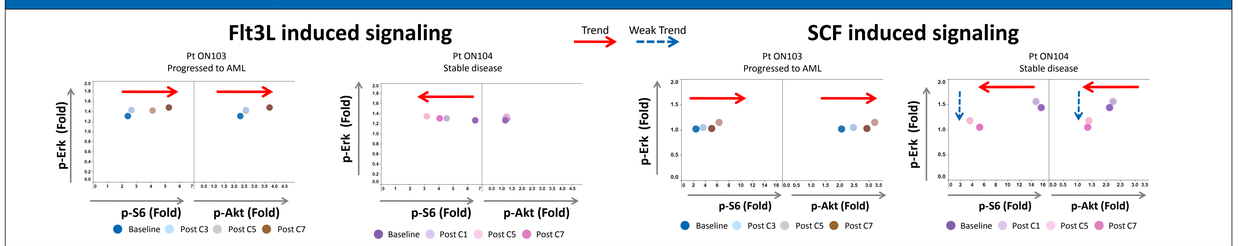
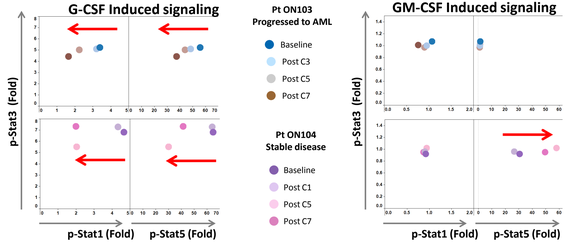


FIGURE 6: CD34+ DIFFERENTIATION SIGNALING RESPONSE WAS MORE SIMILAR BETWEEN THE TWO PATIENT SAMPLES



		Proliferation / Survival signaling response		Differentiation response	
	%CD34+ marrow	Flt3L mod	SCF mod	G-CSF mod	GM-CSF mod
ON103 progressed	↑	↑p-Akt	↑p-Akt	↓ p-Stat1	— p-Stat1
		↑p-S6	↑p-S6	↓ p-Stat5	— p-Stat5
ON104 stable	↓	— p-Akt	↓ p-Akt	↓ p-Stat1	— p-Stat1
		↓ p-S6	↓ p-S6	↓ p-Stat5	↑p-Stat5

Not all nodes altered in response across longitudinal sampling
- Flt3L / SCF → p-Erk
- G-CSF / GM-CSF → p-Stat3

While G-CSF → p-Stat1 signaling decreased across the time course, the response was the same in both patient samples

CONCLUSIONS

- SCNP analysis of BMMC samples from 2 MDS patients, one of whom progressed to AML, established that measurement of signaling networks is feasible in these samples.
- During the course of treatment, increased CD34+ cell counts correlated with progression to AML.
- The analysis of patient samples in this longitudinal study, under therapeutic pressure of treatment with ON 01910.Na, identified altered cellular signaling that contrasted in the 2 patients.
- Over the 7 cycles of treatment, signaling nodes in CD34+ BMMC showed differential responses, between the 2 patient samples, to external modulators.
  - Signaling associated with proliferation and survival showed strong discrimination in 4 nodes, with increased response correlating with progression to AML.
    - Flt3L→ p-S6; Flt3L → p-Akt; SCF → p-S6; SCF → p-Akt
  - Signaling associated with cellular differentiation discriminated only through the GM-CSF → p-Stat5 node, which increased longitudinally in the patient with stable disease.
- Validity of these observations will be evaluated by studying additional samples from same study.

Acknowledgements

We thank all patients who have donated samples for this investigation.

Disclosures

* Denotes Nodality, Inc. employees and stockholders.