A Novel Nano-immunoassay (NIA) Reveals Inhibition of PI3K and MAPK Pathways in CD34+ Bone Marrow Cells of Patients with Myelodysplastic Syndrome (MDS) Treated with the Multi-Kinase Inhibitor ON01910.Na (Rigosertib)

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Abstract

The ability to quantify changes in protein activity in a clinical setting is important for the development of therapeutics that target cancer signaling pathways. We have developed a sensitive nano-immunoassay (NIA, Nanopro1000) to quantify un-, mono- and multi- phosphorylated isoforms of proteins using only 2nL of lysate from patient specimens. We used NIA to confirm that the styryl sulfone mitotic inhibitor, rigosertib, inhibits multiple kinases *in vitro*, including the PI3K and MAPK signaling pathways. To determine if these changes occur *in vivo*, we used NIA to quantify MEK and AKT isoforms in bone marrow CD34+ cells sampled before and at sequential time points after initiating rigosertib treatment in patients enrolled in our Phase II clinical study of rigosertib in MDS patients with Trisomy 8 or Intermediate-1, 2 or High Risk. We have analyzed 14 specimens from 5 patients. Three patients with marrow complete response or stable disease exhibited a 20% mean decrease in phospho-MEK1 and a 15% decrease in phospho-AKT2. In contrast, two patients whose disease progressed exhibited a 15% mean increase in phospho-MEK1 and an 18% increase in phospho-AKT2. Our results suggest that a possible mechanism of action of rigosertib in MDS patients might be through the inhibition of both the PI3K and the MAPK pathways, and raise the hypothesis that the drug may preferentially target specific phosphorylated isoforms within each pathway. We have shown that NIA can be used to measure isoforms of phospho-MEK and phospho-AKT as potential biomarkers of rigosertib activity in MDS.

Goals

- Develop NIA to distinguish and measure changes in proteins in bone marrow CD34+ cells from human patients with MDS upon ON01910.Na treatment
- Distinguish changes in specific isoforms of signaling proteins in MAPK and PI3K pathways
- Correlate proteomic response with clinical outcome

Methods

Schematic of NIA Protein Detection

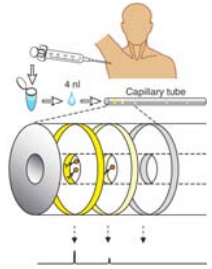

Figure 1. Automated capillary-based system. Lysate undergoes isoelectric focusing in capillary tube. Protein is fixed to capillary wall, and detected using primary antibody. Secondary antibody conjugated to HRP. Readout is a chemiluminescent intensity tracing in relative light units.

Bone marrow can be Stored Overnight at 4º C prior to CD34+ isolation for NIA Analysis

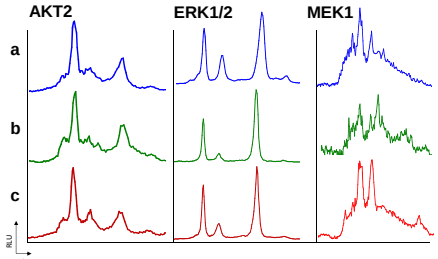

Figure. 2. Mononuclear cells [MNC] are isolated from whole bone marrow (BM) with a ficoll gradient, then CD34+ cells are isolated using magnetic beads. (a) All steps occurred immediately after collection, (b) BM was stored overnight at 4º C, then the next day, MNCs were isolated, followed by CD34+ cell isolation, or (c) MNC were isolated immediately, then stored overnight at 4º C, then CD34+ cells were isolated the next day.

Results

p- MEK1 and p-AKT2 Decreased in CD34+ Cells From Pts who Responded to Rigosertib

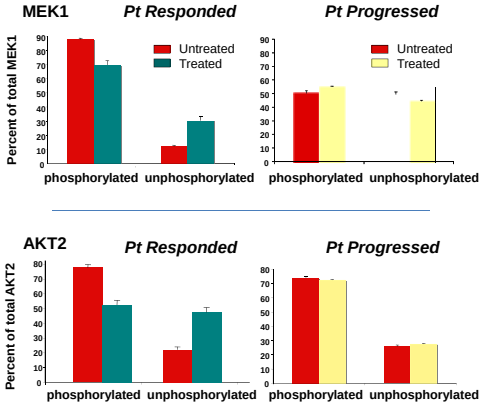

Figure 3. NIA analysis of CD34+ cells from 5 patients was performed in triplicate. Representative examples are shown.

NIA Distinguished 4 Phosphorylated isoforms of AKT2

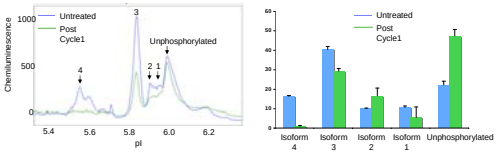

Figure 4. Isoelectric separation of proteins was able to resolve different charged phospho-isoforms from one another. The phospho-isoforms were further confirmed by treatment with phosphatase: only the unphosphorylated peak remained after phosphatase treatment.

Results

Signaling Inhibition was Maintained for 5 months

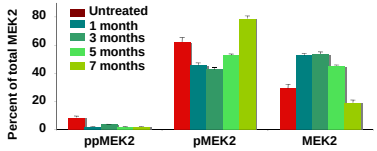

Figure 5. Blood buffy coat cells were drawn at serial time points for this patient, and analyzed using NIA

Changes of phosphorylated AKT2 and pAKT2 Isoform 3 Correlate to Clinical Outcomes

Patients	Clinical response	% change in phospho-AKT2 levels[a]	% change in phospho-AKT2 isoform 3 levels[a]
101	Stable disease	19% decrease	22% decrease
104	Marrow CR	15% decrease	36% decrease
105	Marrow CR	12% decrease	25% decrease
111	Progressive disease	2% decrease	10% decrease
121	Progressive disease	18% increase	22% increase

Summary

We have developed the use of NIA to profile signaling proteins in CD34+ cells

- We have evidence that ON01910.Na decreases in AKT and MEK1 signaling in a subset of patients
- Decreases in these proteins appear to correlate with clinical response
- Measurements of specific phospho-isoforms and percentage of phosphorylation might be developed as biomarkers for clinical outcome

We now aim to develop proteomic biomarkers to confirm the mechanism of biologic response and predict clinical response of MDS to ON01910.Na in a larger cohort of patients

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