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 after rigosertib treatment at 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.

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.

Results

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

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) MNCs were isolated immediately, then stored overnight at 4º C, then CD34+ cells were isolated the next day.

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

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

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

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 phosphorylated 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.