Methods were used: anticipating possible resistance to SAR302503 in particular.

Two approaches were used to generate resistant clones: Selective pressure.
- HEL and SET-2 cells were treated with SAR302503 at concentrations in excess of 2-, 4-, and 10-fold the IC₅₀ for each cell line for up to 8 or 12 weeks to induce resistant clones. Ba/F3.JAK2V617F cells were similarly treated with either SAR302503 or INCB18424.
- Multi-step mutagenesis (SMaDIG) induced mutagenesis followed by compound selection.
- HEL and SET-2 cells were treated with the chemical mutagenENU for 24 hours to induce mutagenesis. Cells were then incubated with SAR302503 at concentrations in excess of 2-, 4-, and 50-fold the IC₅₀ for each cell line to select for enhanced HEI, and SET-2 cells.

Analysis of resistant clones
- Sequencing of JAK2ex2a from the resistant clones was carried out to determine the presence of any mutations.
- In a selected number of SET-2 and HEL resistant clones, signaling and proliferation analyses were performed.

Impact of SAR302503 resistance on JAK/STAT signaling

- Enhanced JAK/STAT signaling in the SAR302503-resistant SET-2 clones was efficiently inhibited at high (1–10 µM) SAR302503 concentrations (Figure 7). The same was true of enhanced MAPK signaling.
- Similar results were observed in SAR302503-resistant HEL clones.

Antiproliferative activity

- SET-2 clones resistant to SAR302503 following 8 weeks of selective pressure were approximately 10-fold less sensitive to SAR302503 than the parental SET-2 cells and were also less sensitive to INCB18424 (Figure 4).

Conclusions
- Cells expressing the JAK2V617F mutation are more prone to develop resistance to INCB18424 than to SAR302503 after treatment with concentrations 4- or 10-fold over their respective IC₅₀ levels.
- Resistance does not arise in cells treated with concentrations of SAR302503 above 3 µM, even after prolonged treatment (up to 12 weeks).
- Resistance to SAR302503 in the patient-derived cell lines HEL and SET is accompanied by hyperactivation of the JAK/STAT pathway in the absence of JAK2 secondary mutations.
- Resistance to SAR302503 in Ba/F3.JAK2V617F cells results from the JAK2V617F mutation.
- Hyperactive JAK/STAT signaling in resistant clones can still be effectively inhibited by clinically achievable concentrations (250–500 ng/mL) of SAR302503.
- Based on the studies shown here, acquired resistance in SAR302503 patients in unlikely because the concentrations of free SAR302503 reached and sustained in the patient (2500 ng/mL) of SAR302503, an anti-pleiotropic daily dose are estimated to be high enough to efficiently inhibit enhanced JAK/STAT activity.

References
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