Anticancer Activity of a Highly Potent Small Molecule Tubulin Polymerization Inhibitor, AB8939

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We have identified the small chemical molecule AB8939 as being a structurally novel, synthesized tubulin inhibitor that can circumvent resistance mechanisms known to limit the effectiveness of existing tubulin inhibitors; e.g., P-glycoprotein (Pgp) and myeloperoxidase (MPO) mediated resistance. A series of *in vitro* preclinical studies provide proof-of-concept that AB8939 has broad applicability as a potent anticancer drug, particularly in tumors of hematopoietic and lymphoid tissues, including acute myeloid leukemia (AML).

Regarding mechanism of action, x-ray crystallography demonstrated that AB8939 binds to the colchicine-binding site on the beta-subunit of tubulin. Cell cycle arrest in the G2/M phase was evaluated using HCT116 cells (a human colorectal tumor), treated at various concentrations of AB8939 for 24 hours. It was seen that AB8939 produced a strong mitotic arrest at the sub-micromolar concentration range (90% of cells in G2/M phase at 10 nM), which was of comparable strength to that of established microtubule targeting agents, each at a concentration of 100 nM. Additional assays using cytarabine (Ara-C) resistant MOLM14 AML cells confirmed this activity, also demonstrating dose dependent (2 to 20 nM) G2/M phase cell cycle arrest in patient-derived AML blasts and that G2/M cell cycle arrest lead to cellular death by apoptosis at nanomolar concentrations.

The effect of AB8939 (100 nM) on the integrity of the microtubule and actin networks was tested in 3T3NIH cells (murine embryonic fibroblast cell line). AB8939 induced a rapid (within 1 hour) and radical destabilization of the microtubule network but did not affect the actin network. Similarly, destabilization of the microtubule network was observed in human primary cardiomyocytes and primary human lung fibroblast cells treated for 24 hours at 10 to 1000 nM AB8939. Further *in vitro* analysis showed that AB8939 produces a direct and potent, dose-dependent depolymerization effect (50% inhibition of *in vitro* microtubule polymerization at around 1 µM, with 100% inhibition at >5 µM).

The potential of AB8939 to overcome resistance to chemotherapeutic agents in Pgp-dependent

multidrug-resistant cell lines was assessed using the drug-sensitive human sarcoma cell line MES-SA (parental) and its multidrug-resistant counterparts MES-SA/MX2 and MES-SA/Dx5 in a 6-day proliferation/survival assay. AB8939 efficiently inhibited each of these cells with an IC_{50} ≤10 nM. By comparison, the MES-SA/MX2 and MES-SA/Dx5 cell lines were highly resistant to the chemotherapeutic agents of doxorubicin and vincristine, as compared with the effect on parental cells (IC_{50} <1.5 – 2.0 µM versus 20 nM, respectively). Additional tests showed that AB8939 is a very poor substrate of Pgp efflux pump, comparable with combretastatin-4, and therefore has the potential to overcome multidrug resistance in cancer patients.

The anti-proliferative activity of AB8939 in various hematopoietic tumors and solid tumors was evaluated using a colorimetric cell proliferation and viability assay. AB8939 produced good anti-tumor activity after 72 hours (IC_{50} of ≤50 nM) in 19 hematopoietic tumor cell lines tested, including AML (3 cell lines), B cell lymphoma (8 cell lines), T cell lymphoma (6 cell lines), and multiple myeloma (2 cell lines). AB8939 also showed good anti-tumor activity after 6 days (IC_{50} of ≤10 nM) in several solid tumor cell lines, including breast, colon, glioblastoma, head and neck, lung, kidney, melanoma neuroblastoma, ovary, pancreas and prostate cell lines.

The therapeutic potential of AB8939 in refractory/resistant AML was investigated further on doxorubicin-resistant AML cell lines (HL60 and U937), doxorubicin being a commonly used AML induction drug and Pgp substrate. AB8939 produced a strong anti-proliferative effect in both cell lines whereas both were resistant to doxorubicin, thus demonstrating AB8939's potential to overcome refractory/resistant AML. Notably, HL60 and U937 are respectively MPO-positive and MPO-negative, indicating that unlike vinca alkaloids (e.g. vincristine or vinblastine) AB8939 it is not deactivated by this myeloid enzyme.

These data show that AB8939 is a prolific and highly potent (nanomolar concentrations) Pgp-independent, next-generation microtubule-destabilizer drug for cancer therapy; in particular, difficult to treat hematopoietic tumors such as relapsed/refractory AML.

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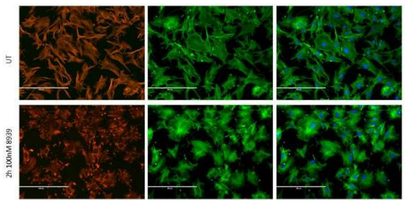
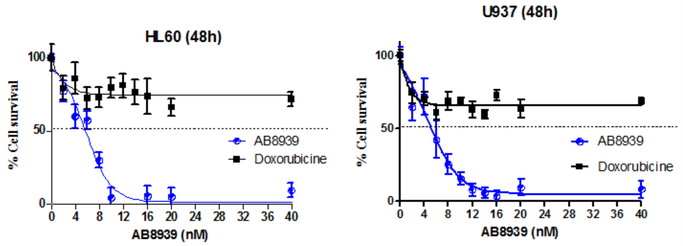
AB8939 Mechanism of Action

AB8939, A SMALL MOLECULE TUBULIN POLYMERIZATION INHIBITOR, HAS BROAD APPLICABILITY AS A POTENT ANTICANCER DRUG, PARTICULARLY IN TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES, INCLUDING AML

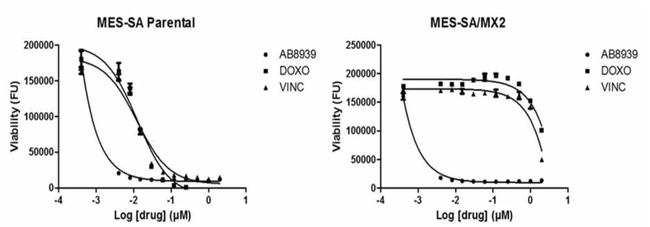
- AB8939 produced good anti-tumor activity (IC_{50} of ≤50 nM) in 19 hematopoietic tumor cell lines (after 72 hours) and several solid tumor cell lines (after 6 days), including:
  - Acute Myeloid Leukemia (AML) (3 cell lines)
  - B cell lymphoma (8 cell lines)
  - T cell lymphoma (6 cell lines)
  - Multiple myeloma (2 cell lines)
  - Breast, colon, glioblastoma, head and neck, lung, kidney, melanoma neuroblastoma, ovary, pancreas and prostate cell lines.
- *In vitro,* AB8939 produces strong mitotic arrest via destabilization of the microtubule network by binding to the colchicine site and is capable of circumventing resistance mechanisms; e.g. myeloperoxidase (MPO) and P-glycoprotein (Pgp).
  - AB8939 induces cell cycle arrest in the G2/M phase at the sub-micromolar concentration range
  - AB8939 induces microtubule depolymerization in intact cells
  - AB8939 directly inhibits tubulin polymerization (µM concentration range) and in a dose-dependent manner
  - AB8939 overcomes multidrug resistance (not a substrate for Pgp; not deactivated by MPO enzyme) including important components of AML induction therapy (i.e. cytarabine and anthracyclines).



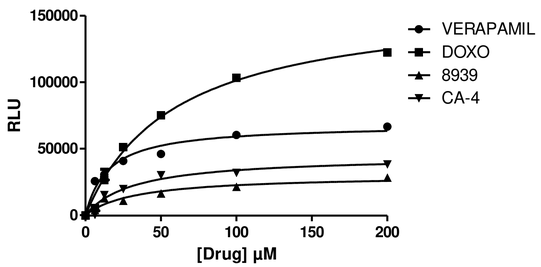
AB8939 induces microtubule depolymerization in intact cells. Immunofluorescent staining of microtubules (red) and actin (green) filaments in NIH 3T3 cells following 2 h AB8939 treatment



AB8939 produced a strong (nanomolar sensitivity) anti-proliferative effect in doxorubicin-resistant AML cell lines (HL60 and U937)



AB8939 overcomes multidrug resistance. AB8939 efficiently blocks proliferation of the Pgp-overexpressing, drug-resistant (mitoxantrone, doxorubicin, vincristine) MES-SA/MX2 cell line in a 6-day proliferation/survival assay.



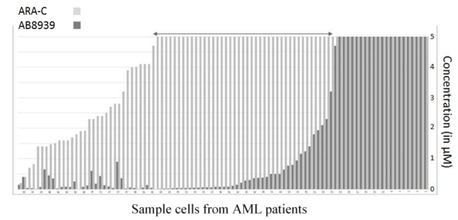
Stimulation of Pgp ATPase activity by AB8939 compared with substrates of Pgp (doxorubicin and verapamil) show that AB8939 is not a substrate of Pgp (combretastatin A-4 is Pgp-negative control)

Assessment of AB8939 in Acute Myeloid Leukemia (AML)

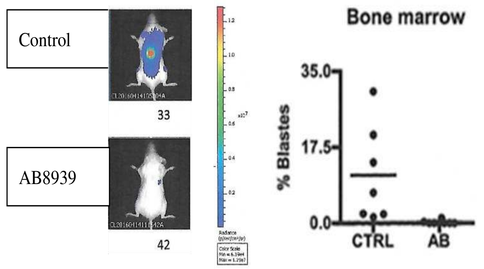
AB8939 HAS BROAD ANTI-PROLIFERATIVE ACTIVITY ACROSS THE ENTIRE RANGE (M0–M7) OF AML SUBTYPES

AB8939 HAS POTENTIAL TO IMPROVE THE TREATMENT OF REFRACTORY/RELAPSE AML

- The potential of AB8939 to overcome Ara-C resistance (IC_{50} >5 µM) was demonstrated in proliferation assays (99 AML patient samples)
  - 66% of Ara-C-resistant blasts were sensitive to AB8939
  - 69% of blasts had nanomolar sensitivity (IC_{50} ≤ 500 nM)
  - 44% of blasts were very sensitive (IC_{50} ≤ 100 nM)



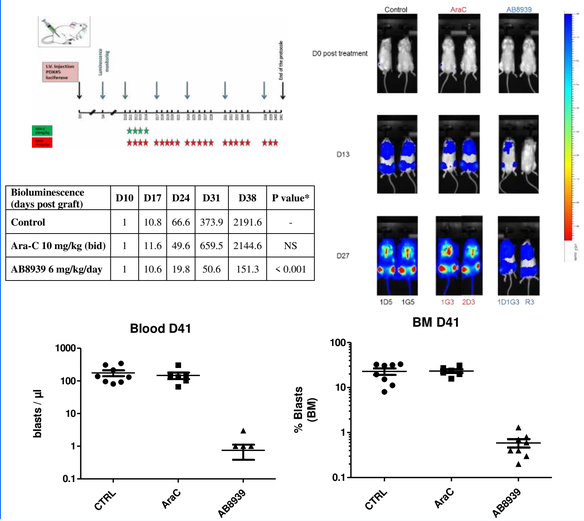
Sample cells from AML patients



- AB8939 eradicates blasts from bone marrow in an AMKL26 PDX mouse model
  - At the end of the 3-week AB8939 treatment period (2 mg/kg i.v. 3d ON / 4d OFF for 2 weeks, then 5 mg/kg 3d ON / 4d OFF for 1 week), blast detection in bone marrow was performed via bioluminescence imaging
  - AB8939 showed strong anti-leukemic activity with near eradication of blasts. AB8939 was well-tolerated with no toxicity-related deaths and no impact on animal body weight or behavior.
  - No blasts could be detected in 6 / 8 mice treated with AB8939.

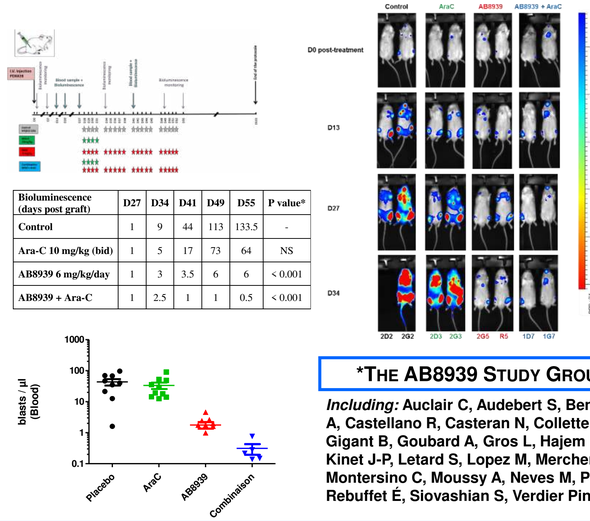
- Therapeutic potential of AB8939 in AML demonstrated *in vivo* using an Ara-C resistant (IC_{50}~8 µM) PDX model

Single agent AB8939: significant decrease in concentration of blasts in blood, bone marrow and tumor growth



Bioluminescence (days post graft)	D10	D17	D24	D31	D38	P value*
Control	1	10.8	66.6	373.9	2191.6	-
Ara-C 10 mg/kg (bid)	1	11.6	49.6	659.5	2144.6	NS
AB8939 6 mg/kg/day	1	10.6	19.8	50.6	151.3	< 0.001

AB8939 plus Ara-A: significant decrease in disease burden (D55 post graft; D27 treatment)



Bioluminescence (days post graft)	D27	D34	D41	D49	D55	P value*
Control	1	9	44	113	133.5	-
Ara-C 10 mg/kg (bid)	1	5	17	73	64	NS
AB8939 6 mg/kg/day	1	3	3.5	6	6	< 0.001
AB8939 + Ara-C	1	2.5	1	1	0.5	< 0.001

*THE AB8939 STUDY GROUP

Including: Auclair C, Audebert S, Benjahad A, Castellano R, Casteran N, Collette Y, Gigant B, Goubard A, Gros L, Hajem B, Kinet J-P, Letard S, Lopez M, Mercher T, Montersino C, Moussy A, Neves M, Pez D, Rebuffet É, Siovashian S, Verdier Pinard P

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COI: AB8939 is developed by AB Science (ABS). MH, AG and CM are employees of ABS. OH and PD are consultants and shareholders of ABS.