Small Molecule Inhibitor of CBFβ-SMMHC for Inv(16) Acute Myeloid Leukemia (AML)

Inventor: John H. Bushweller
Unmet need for Inv(16) Subtype of AML

- ~35,000 new cases/year worldwide (~10% of all AML cases). ~2,000 new cases/year in US
- Patient cohort is easily identified by cytogenetics analysis which is standard for Leukemia patients
- Currently available treatments
  1. Traditional cytotoxic chemotherapy (anthracycline e.g. daunorubicin + cytarabine)
     - Long-term side effects
     - Poorly tolerated by older patients
     - High rates of relapse
       - 42% of Inv(16) patients will relapse within 3 years
       - Substantial burden on the patients and the health care system
  2. Bone marrow transplant
     - Requires a compatible donor
     - High risk due to complete ablation of the immune system
- Currently available treatments are expensive; $100,000/patient for chemo, $120,000/patient for transplant
Mechanism of Inv(16) AML: unique CBFβ-SMMHC fusion

CBFβ-SMMHC binds RUNX1 and blocks normal RUNX1 driven gene transcription, leading to Inv(16) leukemia.
Novel CBFβ-SMMHC targeted small molecule for Inv(16)

- Original hit identified from FRET-based screen for disruptors of CBFβ-SMMHC/RUNX1 Runt domain binding. Optimized to obtain Al-10-49.
- IC_{50} in FRET assay for CBFβ-SMMHC/RUNX1 Runt domain binding = 260 nM
- Isothermal titration calorimetry K_d = 170 nM

Al-10-49 restores RUNX1 occupancy in the genome and RUNX1 driven gene expression.

Al-10-49 selectively disrupts CBFβ-SMMHC binding to RUNX1 but not wildtype CBFβ binding to RUNX1 – Extraordinary selectivity.

Advantages over existing therapy

- **Selectivity**
  - Targeting the oncogenic lesion that drives the disease rather than non-selectively targeting all rapidly growing cells with traditional chemotherapy

- **Safety**
  - Induces apoptosis in Inv(16)/RAS(G12D) cells, so killing leukemic cells, but not normal hematopoietic cells
  - No demonstrated toxicity thus far

- **Prevent relapse**

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*Al-10-49 induces differentiation in Inv(16) leukemia initiating cells thus addressing minimal residual disease (MRD).*
AI-10-49 shows efficacy in a CBFβ-SMMHC/RAS(G12D) mouse model of inv(16) leukemia with 10 days of once daily dosing (207.5 mg/kg).

AI-10-49 shows efficacy against human inv(16) patient samples but not normal human bone marrow cells.

AI-10-49 reduces MYC expression, thereby reducing a MYC driven gene expression program.

ADMET properties of AI-10-49

- No toxicity in hematopoietic compartment nor in major organs in mice after 1 week of daily dosing

- Profiled for toxicity using Eurofins Panlabs LeadProfilingScreen of 68 possible toxicity targets
  - Profiled at 10 μM
  - Inactive against 61/68 targets
  - IC50 ≥ 8 μM for 7 targets

- Half-life in human liver microsome assay
  - 8 minutes

- Human plasma protein binding
  - 88% at 1 μM
  - 95% at 10 μM

- Developed two formulations with chemical and thermodynamic stability, which disperse well in simulated gastric and intestinal fluid
AI-10-49 + Doxorubicin

In vivo test of combination showed synergistic effects on:

- Reducing the white blood cell
- Reducing c-Kit⁺ which is an enriched fraction containing the leukemic cells
- Reducing spleen weight and cell number
AI-10-49 + Doxorubicin

A clear synergistic effect on reducing Lin-Sca1-C-kit+ cell population which is enriched in the leukemia initiating cell (LIC) or leukemia stem cell (LSC) population, whereas only a modest effect was seen with AI-10-49 alone and no effect was seen with Dox alone.

These results argues that the combination effectively target the reservoir of leukemic cells that drives relapse.
Relevant Publications & Intellectual Property

- Publications

- IP (UVA Tech IDs: BUSHWEL- INV16, BUSHWEL-DOX)
  - US 8,748,618, expired in Oct. 2030
  - US 9,221,764, expired in May 2030
  - US 9,926,290, expired in May 2030
  - EP 10775546.4, pending
  - PCT/US2017/044124, pending
Ongoing development and future plan

- Current medicinal chemistry efforts focused on improving potency and ADMET properties
- Developing second generation compounds
- In vitro and in vivo toxicology
- In vivo PK/PD and bioavailability characterization