The microtubulin-disrupting drug BNC105 is a potent inducer of apoptosis in AML patient samples

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Abstract #1881

BNC105 is a high potency inducer of apoptosis

BNC105 is a Phase II potent and highly selective disruptor of micro-vasculature in solid tumors leading to the rapid onset of hypoxia and necrosis as the tumor becomes oxygen and nutrient starved.

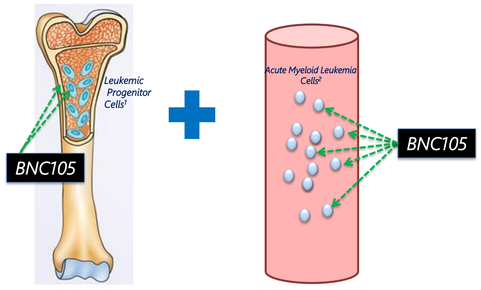
BNC105 targets the colchicine-binding site on tubulin causing chronic disruption of adhesion molecules particularly in neo-vasculature and was developed to be best-in-class with high specificity to actively dividing cells.

It has one of the largest therapeutic windows of its class of vascular disruptors and has been shown to also have direct cytotoxic activity on tumor cells. It is this highly tumor-specific mechanism of action that has also positioned BNC105 as a therapeutic with high potential in the haematological cancer setting.

Previous studies of BNC105 when used to treat chronic lymphocytic leukemia (CLL) patient samples have shown that treatment results in the activation of c-Jun N-terminal kinase (JNK), phosphorylation of ATF2, and the induction of ATF3 and Noxa, which lead to acute apoptosis. These findings led to the commencement of a Phase I trial of BNC105 combined with standard of care Ibrutinib in patients with chronic lymphocytic leukemia (NCT03454165).

Here we investigate the effect of BNC105 treatment on Acute myeloid leukaemia (AML), a disease that currently has limited treatment options. To assess the utility of BNC105 therapy in this setting, AML cell lines and patient samples representing different subtypes, including the high risk FLT3-ITD subtype, were treated with BNC105 to assess the therapeutic potential.

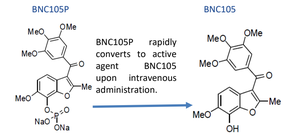
BNC105 accesses both Bone Marrow and Peripheral Blood



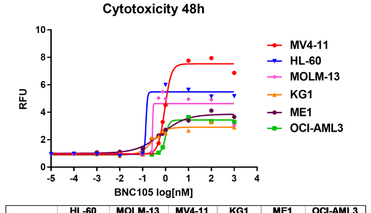
BNC105 kills the leukemic progenitor cells potentially reducing recurrence of the leukemia

BNC105 induces activation of death proteins in AML cells in the circulation resulting in a direct cell kill

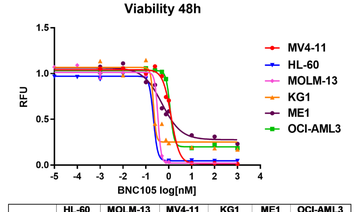
BNC105 is administered as its disodium phosphate ester pro-drug form BNC105P. After administration it is rapidly cleaved to its active form BNC105



BNC105 has sub-nanomolar activity on an AML cell line panel



	HL-60	MOLM-13	MV4-11	KG1	ME1	OCI-AML3
EC50	~ 0.1401	~ 0.2729	0.9325	0.2728	0.6704	0.9949



	HL-60	MOLM-13	MV4-11	KG1	ME1	OCI-AML3
IC50	0.2189	0.3397	1.286	0.2348	1.036	1.263

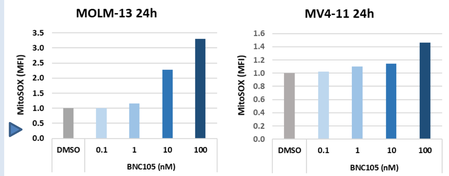
A panel of AML cell lines were exposed to BNC105 *in vitro* for 48 hours and assessed for cytotoxicity (CellTox Green) and viability (CellTiter-Blue.)

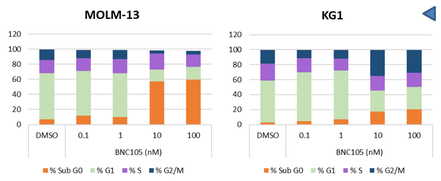
IC_{50} values reported for all subtypes including FLT3-ITD cells were in the sub-nanomolar range.

Cell line	Details	FAB Subtype
MV4-11	MLL-AF4 AML (FLT3-ITD)	M5
MOLM-13	MLL-AF9 AML --> after MDS (RAEB) (FLT3-ITD)	M5a
ME-1	inv(16)	M4eo
KG-1	AML –erythroleukemia that developed into AML at relapse	M0/M1
HL-60	AML (APML) with PML-RARA	M3 --> M2
OCI-AML3	AML - NPM mutation, DMNT3A R882C	M4

Investigating the mechanism by which BNC105 targets AML cells

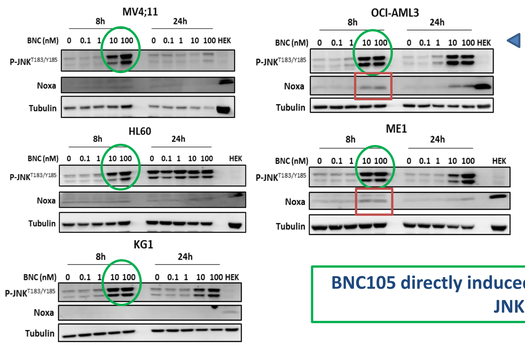
The production of reactive oxygen species (ROS), cell cycle distribution and cell signaling by western blot were all assessed after treatment.
ROS levels were measured in the 6 cell lines after 24 hours of treatment with a range of doses of BNC105. Mitochondria superoxide anion levels were measured by flow cytometry using MitoSOX.
MV4-11 and MOLM-13 showed a dose response to the drug with increased mitochondria superoxide anion levels observed at 24 hours.





There were different patterns of cell cycle distribution after treatment with BNC105 for 24 hours. MV4-11, MOLM-13, KG1 and HL-60 showed increased levels of apoptotic cells (sub-G0/G1) concomitant with decreased levels of cells in G0/G1. In contrast, increased sub-G1 cells were not observed in ME1 and OCI-AML3 which showed increased accumulation of cells in the G2/M phase of the cell cycle, consistent with G2/M arrest in response to treatment with BNC105. KG1 and HL-60 show increased sub-G0/G1 as well as some increase in the level of cells in G2/M.

BNC105 directly induced cell cycle arrest in AML cell lines

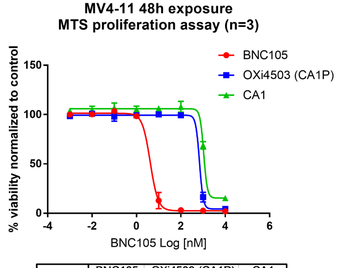


To further investigate the mechanism by which BNC105 targets these AML cell lines, western blot analysis of apoptosis related proteins was performed.
p-JNKT183/Y185 is induced in all cell lines with 10nM BNC105 at 8 hours.
Noxa induction is observed in OCI-AML3 and ME1 lines treated with 10nM BNC105 at 8 hours.

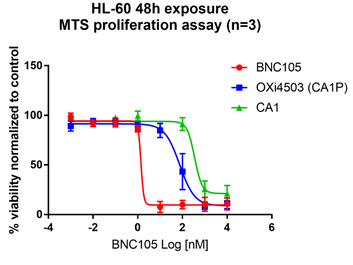
BNC105 directly induced pro apoptotic proteins Noxa and p-JNK in AML cell lines

BNC105 is more potent than competitors on AML cell lines

BNC105 was more potent that OXi4503 (CA1P) or the active form CA1 when HL-60 and the Flt3-ITD cell line MV4-11 cells were exposed *in vitro* for 48 hours. Both cell lines were seeded at 10000 cells/well. Cell viability was measured using MTS reagent.
Data is from three combined experiments with each point representing Mean ± SEM, n=3.



	BNC105	OXi4503 (CA1P)	CA1
IC50	4.173	~ 692.8	~ 1071

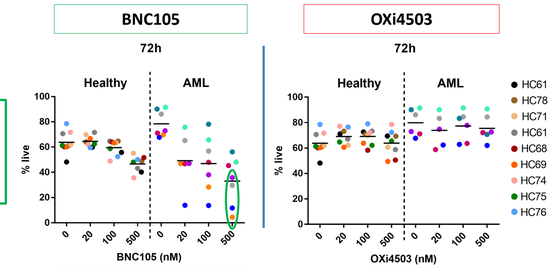


	BNC105	OXi4503 (CA1P)	CA1
IC50	~ 1.395	75.61	350.5

BNC105 is potent in killing AML patient cells

AML patient samples obtained from the South Australian Cancer Research Biobank (SACRB) were exposed to BNC105 at clinically relevant doses for up to 72 hours and cellular viability and apoptosis induction were assessed by Annexin V/7AAD staining. BNC105 significantly decreased viability in a dose and time dependent manner, including the FLT3 mutant subtype patient samples. In comparison, bone marrow mononuclear cells from healthy controls were much less affected by BNC105.

3 out of 4 of the highest responding patient cell isolates had a FLT3 mutation

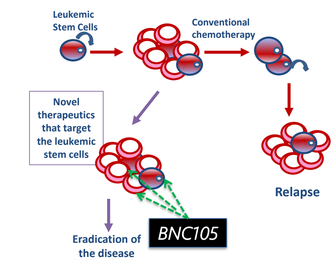


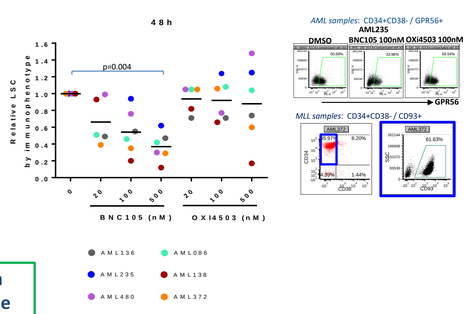
BNC105 is potent in killing patient AML cells

OXi4503 has no effect on patient AML cell viability at the concentrations tested

BNC105 kills AML progenitor cells at nanomolar concentrations

Effects of BNC105 on the leukemic stem cell (LSC) phenotype population were also investigated. The LSC-containing population, measured by CD34+/CD38- and GPR56+ or CD93+ staining, was targeted by BNC105 in all AML patient samples tested.





BNC105 is more potent than Oxi4503 in killing patient progenitor AML cells at the concentrations tested

Clinical investigation of BNC105 for treatment of AML is warranted

- **We have demonstrated *ex vivo* that BNC105 is a high potency inducer of apoptosis and cell death in patient AML cells.**
- **BNC105 targets both peripheral acute leukemic and leukemic progenitor cell populations potentially reducing the potential for recurrence.**
- **BNC105 was shown to be more potent than OXi4500 (CA1) and OXi4503 (the phosphate prodrug of CA1). OXi4503, a vascular disrupting agent, is currently in a Phase Ib/II clinical trial in combination with Cytarabine (NCT0256301) and has achieved FDA Fast Track designation for the treatment of Acute Myeloid Leukaemia (AML).**
- **AML cells can be directly targeted by BNC105 at clinically relevant concentrations. Further clinical investigation of BNC105 is warranted for AML treatment in a patient population with high unmet need.**