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Non clinical pharmacology, pharmacokinetics and safety profiling of CB-103: A novel first-in-class small molecule inhibitor of the NOTCH pathway

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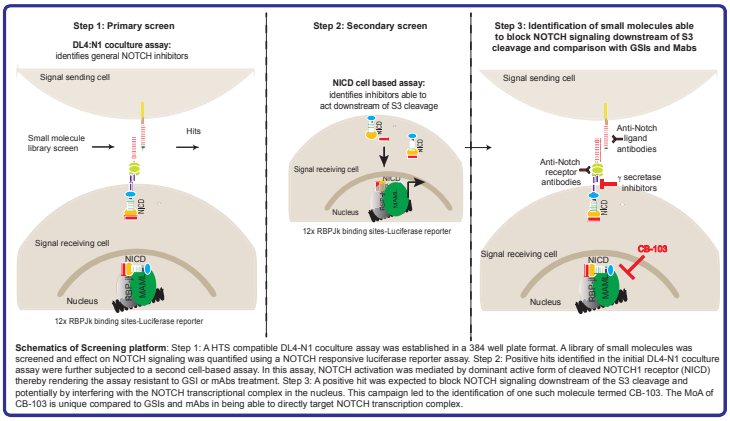
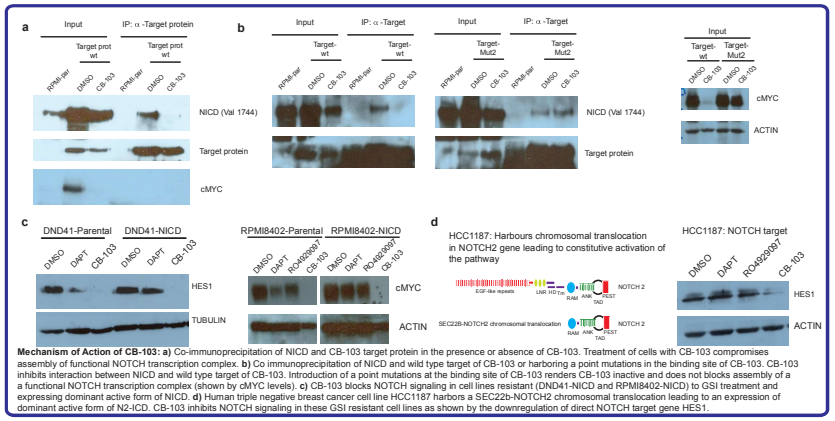
Abstract: NOTCH signaling is a developmental pathway known to play critical roles in the regulation of self-renewing tissues. Aberrant activation of NOTCH signaling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. Over activation of NOTCH in human cancers can be a consequence of over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations leading to constitutive activation of the pathway. Activation of NOTCH and its oncogenic role in various cancers is prognostically relevant with shorter survival seen in patients harbouring these genetic alterations. Given the importance of Notch signaling in human cancers, several therapeutic approaches have been utilized to block NOTCH signaling. Two of these strategies are; a) the use of monoclonal blocking antibodies (mAbs) against NOTCH ligands and receptors and b) the use of small molecule γ-secretase inhibitors (GSIs). In human cancers harbouring NOTCH gene fusion due to chromosomal translocations, the use of mAbs and GSIs will have very limited clinical benefits.

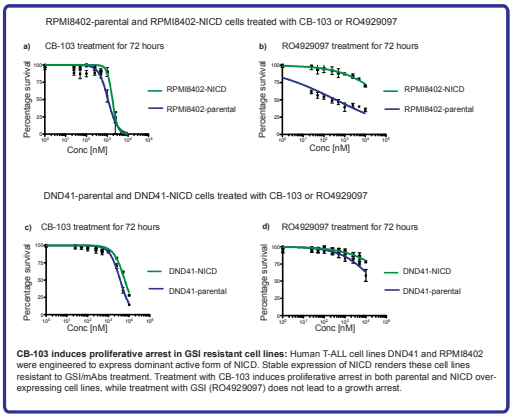
Here we report discovery and characterization of CB-103, a novel first-in-class orally-active small molecule inhibitor, of the NOTCH pathway. CB-103 inhibits NOTCH signaling by targeting the NOTCH transcriptional activation complex in the nucleus. In a GSI/mAb resistant xenograft models of human triple negative breast cancer, human T-ALL and mouse mammary tumor, CB-103 has demonstrated excellent anti-tumor efficacy. In this study we present additional pharmacology, PK data and provide an overview of the good safety and tolerability seen with CB-103 in the non-clinical studies. Based on these findings CB-103 has been selected as clinical candidate to be investigated in a FIM, phase I/IIA clinical study in advanced cancer patients.



Schematics of Screening platform: Step 1: A HTS compatible DL4-N1 coculture assay was established in a 384 well plate format. A library of small molecules was screened and effect on NOTCH signaling was quantified using a NOTCH responsive luciferase reporter assay. Step 2: Positive hits identified in the initial DL4-N1 coculture assay were further subjected to a second cell-based assay. In this assay, NOTCH activation was mediated by dominant active form of cleaved NOTCH1 receptor (NICD) thereby rendering the assay resistant to GSI or mAbs treatment. Step 3: A positive hit was expected to block NOTCH signaling downstream of the S3 cleavage and potentially by interfering with the NOTCH transcriptional complex in the nucleus. This campaign led to the identification of one such molecule termed CB-103. The MoA of CB-103 is unique compared to GSIs and mAbs in being able to directly target NOTCH transcription complex.



Mechanism of Action of CB-103: a) Co-immunoprecipitation of NICD and CB-103 target protein in the presence or absence of CB-103. Treatment of cells with CB-103 compromises assembly of functional NOTCH transcription complex. **b)** Co immunoprecipitation of NICD and wild type target of CB-103 or harboring a point mutations in the binding site of CB-103. CB-103 inhibits interaction between NICD and wild type target of CB-103. Introduction of a point mutations at the binding site of CB-103 renders CB-103 inactive and does not blocks assembly of a a functional NOTCH transcription complex (shown by cMYC levels). **c)** CB-103 blocks NOTCH signaling in cell lines resistant (DND41-NICD and RPMI8402-NICD) to GSI treatment and expressing dominant active form of NICD. **d)** Human triple negative breast cancer cell line HCC1187 harbors a SEC22b-NOTCH2 chromosomal translocation leading to an expression of dominant active form of N2-ICD. CB-103 inhibits NOTCH signaling in these GSI resistant cell lines as shown by the downregulation of direct NOTCH target gene HES1.



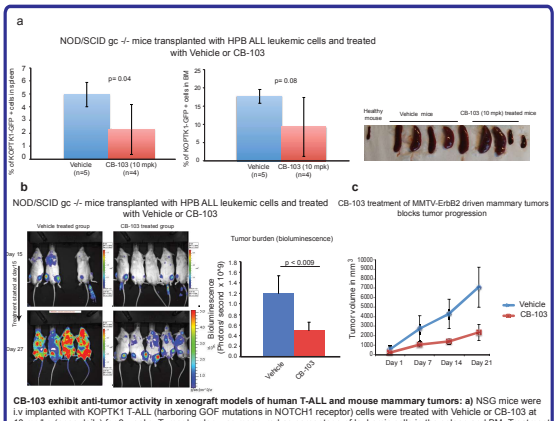
CB-103 induces proliferative arrest in GSI resistant cell lines: Human T-ALL cell lines DND41 and RPMI8402 were engineered to express dominant active form of NICD. Stable expression of NICD renders these cell lines resistant to GSI/mAbs treatment. Treatment with CB-103 induces proliferative arrest in both parental and NICD over-expressing cell lines, while treatment with GSI (RO4929097) does not lead to a growth arrest.

INDICATIONS	CELL LINES	NOTCH STATUS	CB-103 IC50 (μM)	GSI IC50 (μM)	PD MARKERS (Downregulation NICD & target genes)
T-ALL	RPMI8402	Positive	0.7	5	Positive
	RPMI8402-NICD	Positive	1-2	>10	Positive
	DND41	Positive	3	>10	Positive
	DND41-NICD	Positive	5	>10	Positive
	KOPTK1	Positive	1	5	Positive
	CUTL1	Positive	3	>10	Positive
Breast	HCC1187	Positive	5	>10	Positive
Immortalized T cell	Jurkat	Positive	0.1		Positive
Mantle cell lymphoma	REC-1	Positive	1		Positive
Cervical	HeLa	Negative	>100	>100	NA
B lymphoma	RAJI	Negative	>10	>10	NA
	HBL1	Negative	>10	>10	NA

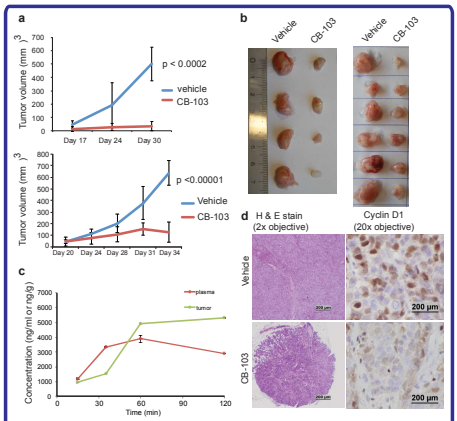
IC50: < 2 μM | IC50: 2 - 10 μM | IC50: >10 μM | IC50: >100 μM

INDICATIONS	CELL LINES	NOTCH STATUS (lit. based)	CB-103 IC50 (μM)	GSI RO4929097 IC50 (μM)	NOTCH PD MARKER
ALL	RS4 11	Positive	0.23	83	Ongoing
AML	P31/FUJ	NA	1.5	>100	Ongoing
	KG1a	NA	1.6	79	Ongoing
	MV4-11	NA	3.3	56	Ongoing
CLL	JVM-3	NA	7.2	65	Ongoing
MM	KMS-12-BM	NA	8.2	>100	Ongoing
Lymphoma	NALM-6	NA	1.1	63	Ongoing
	U-937	Positive	9.4	59	Ongoing
Cervix	C33a	Positive	1.2	>100	Ongoing
Neuroblastoma	SK-N-MC	Positive	1.5	44	Ongoing
Osteosarcoma	SK-ES-1	Positive	1.9	>100	Ongoing
Endometrium	Ishikawa	Positive	3	81	Ongoing
Breast	HCC 1569	Positive	5.3	100	Ongoing
Lung	NCI-H1581	NA	5.5	60	Ongoing

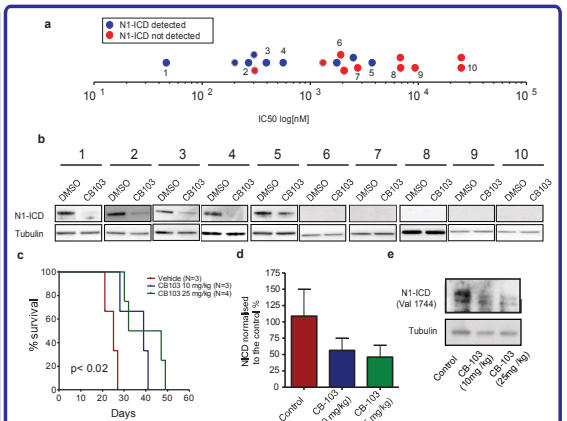
CB-103 exhibit activity in cancer cell lines representing several human cancers: A panel of 136 human cancer cell line was screened for anti-tumor activity of CB-103 in comparison with GSIs (DAPT or RO4929097). Several known NOTCH positive cell lines responded to CB-103 at low μM concentration range and showed superior activity over GSIs.



CB-103 exhibit anti-tumor activity in xenograft models of human T-ALL and mouse mammary tumors: a) NSG mice were i.v implanted with KOPTK1 T-ALL (harboring GOF mutations in NOTCH1 receptor) cells were treated with Vehicle or CB-103 at 10 mg/kg (once daily) for 3 weeks. Tumor burden was measured as percentage of leukemic cells in the spleen and BM. Treatment with CB-103 at low doses leads to a reduction in GFP + human leukemic cells in the spleen and BM as well as reduction of splenomegaly in 50% of the animals. **b)** Human T-ALL cell line HPBALL (harboring GOF mutations in NOTCH1 receptor) was i.v implanted in NSG mice and tumor growth was monitored by BLI measurement. Leukemia bearing mice were treated with vehicle or CB-103 at 25 mg/kg for 2 weeks.BLI measurements show significant reduction in tumor burden in CB-103 treated animals **c)** MMTV-ErbB2 tumors (with over expression of HES1, data not shown) were implanted in FVB mice and treated with vehicle or CB-103 at 25 mg/kg. Tumor volume mesurements showed growth retardation in CB-103 treated animals.



CB-103 inhibits growth of GSI/Mab resistant triple negative breast cancer in xenograft model: NSG mice were sub cutaneously transplanted with HCC1187 (stable for luciferase expression). **a-b)** Treatment of tumor bearing mice with CB-103 (25mg/kg, 2x daily) leads to a growth arrest of triple negative breast cancer as shown by tumor volume measurements. **c).** Accumulation of CB-103 in tumor tissue over blood plasma **d)** CB-103 treatment leads to a downregulation of NOTCH target and proliferation marker cyclin D1.



CB-103 blocks *ex vivo* and *in vivo* growth of PDX models of human T-ALL: a & b) Primary human T-ALL patient samples were grown *ex vivo* and treated with CB-103 for 72 hours. Patient samples positive for accumulation of N1-ICD (**b**), indicating activation of the NOTCH pathway responds to CB-103 treatment at nM range, while patients with no pathway activation responds to CB-103 treatment at high uM range. **c-e)** Primary human T-ALL patient sample was transplanted into immunocompromised mice. Upon establishment of the disease, animals were treated with CB-103 (10 and 25 mg/kg). CB-103 treatment leads to a reduced tumor burden and a prolonged survival as well as downregulation of N1-ICD, indicating pharmacological effect of CB-103.

Conclusions: Cellestia Biotech's drug development program has led to the discovery and development of a novel chemical series of pharmacological inhibitors of the NOTCH pathway for which the current Development Candidate is CB-103. Our studies demonstrate that CB-103 inhibits NOTCH signaling through a unique mechanism of action. CB-103 blocks NOTCH signaling downstream of S3 cleavage of NOTCH receptors by directly targeting NOTCH transcription complex in the nucleus. Due to its novel mechanism of action, CB-103 effectively blocks NOTCH signaling mediated by dominant active forms of NICD, thus enabling application of CB-103 in human tumors driven by GOF mutations in the NOTCH receptors and by chromosomal translocation in NOTCH receptor genes (~9% TNBC) This will allow an application of CB-103 in GSI and mAbs (targeting NOTCH ligands and receptors) resistant human tumors. In PDX model of human T-ALL, CB-103 showed anti-cancer activity in chemorefractory and NOTCH positive patient sample. The response in this setting was accompanied by downregulation of NICD that acts a PD marker for anti-NOTCH activity of CB-103. Cellestia Biotech has successfuly completed CTA/IND-enabling studies and FIM trial is planned during Q3-2017.

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