Cell penetrating single domain antibody (sdAb) SBT-100 binds KRAS & inhibits growth of human cancers with KRAS activating mutations.

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Abstract
1544 / 13

Singh BIOTECHNOLOGY

ABSTRACT

BACKGROUND: Despite nearly forty years of research, scientists have failed to develop a clinically viable therapy against KRAS, one of the deadliest families of cancer-causing proteins. Mutations in KRAS are prevalent amongst the top three most deadly cancer types in the United States: pancreatic (95%), colorectal (45%), and lung (35%). KRAS has been thought to be undruggable due to: 1) its intracellular location and lack of binding pockets for small molecules; 2) the high (pM) affinity of RAS for GTP precludes direct targeting of the nucleotide binding pocket; 3) high intracellular concentrations of GTP (uM) inhibits competition for the nucleotide-binding pocket by small molecules; and 4) possible toxicity. Mutations of KRAS result in it being perpetually turned on to propagate signal down the MAPK pathway. This results in constant production of P-ERK and plays an important role in malignant development. To overcome these challenges, Singh Biotechnology has developed SBT-100 a first in class & best in class novel sdAb that penetrates the cell membrane to bind KRAS to inhibit its GTPase activity.

METHODS: Human cancer cell lines were purchased from ATCC. BIAcore affinity studies were conducted by Precision Antibody. KRAS GTPase assay was purchased from Promega. Levels of P-ERK were determined using western blots. In vitro cell growth suppression was tested with MTT assay. Athymic nude mice for xenograft studies were purchased from Envigo.

RESULTS: SBT-100 binds KRAS with K_D=10^{-9} and KRAS(G12D) with K_D=10^{-7} as demonstrated by BIAcore affinity assay. Both SBT-100 and SBT-102 significantly inhibit KRAS GTPase activity in vitro and inhibition is comparable to polyclonal antibody to KRAS. Growth of MDA-MB-231 cells with KRAS(G13D) mutation and PANC-1 cells with KRAS(G12D) mutation are significantly decreased in the MTT assay when incubated with SBT-100. Additionally, same cell lines have significantly decreased P-ERK expression when cultured with SBT-100. Xenograft studies demonstrate significant growth suppression of MDA-MB-231 and PANC-1 when treated with SBT-100 in vivo.

CONCLUSION: SBT-100, crosses the cell membrane, binds to KRAS intracellularly and its most common mutant with nanomolar affinity, inhibits KRAS GTPase activity, downregulates P-ERK signaling, and suppresses the growth of cancers cells in vitro and in vivo without showing any toxic effects.

KRAS: Role in Cancer

RAS family: HRAS, NRAS, KRAS

KRAS, part of a family of proteins commonly mutated in cancer, is one of the most desirable drug targets in the pharmaceutical industry.

Ras proteins play a role in about 30% of all human cancers.

- 95% Pancreatic Adenocarcinomas (KRAS)
- 95% Brain: Glioblastomas (KRAS)
- 45% Colorectal (KRAS)
- 35% Lung Adenocarcinomas (KRAS)
- 15% Acute Myeloid Leukemia (NRAS)
- 15% Melanoma (NRAS)
- 10% Bladder (HRAS)

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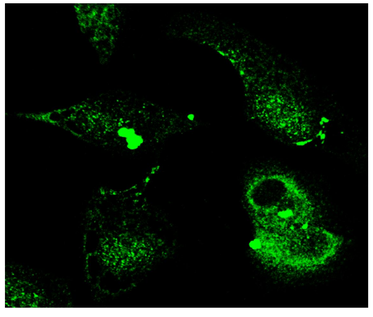
Source: 2016 National Cancer Institute

SBT-100 Intracellular Binding

Positive staining in cytoplasm. Confocal Image of anti-VHH IFA, SBT-100, 6hr in MDA-MB-231 (TNBC) cells.

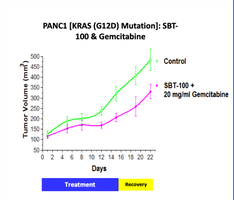


SBT-100: Inhibits KRAS Mutant Cancer Cell Lines

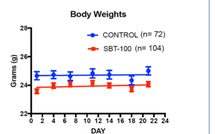
Cancer	Human Cancer Cell Line	% Inhibition in 3 days (100 ug/ml)
Pancreatic	PANC-1	85% (p<0.001)
TNBC	MDA-MB-231	89% (p<0.001)
Prostate	DU-145	92% (p<0.001)

In Vitro Growth Inhibition Determined by MTT Assay

SBT-100 *In Vivo* Efficacy in Pancreatic Cancer Cells: In Combination with Gemcitabine

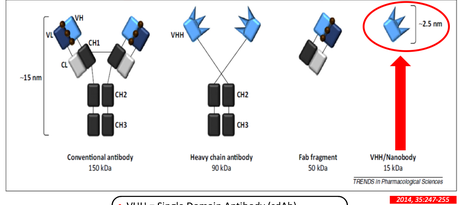


% Inhibition of Control				
Group	Day 12	Day 15	Day 19	Day 22
20 mg/kg Gemcitabine	19.51	23.17	23.26	14.93
5 mg/kg SBT-100 BID	20.7	27.29	18.37	19.17
5 mg/kg SBT-100 + 20 mg/kg Gemcitabine	28.83	35.48	36.37	31.52



No Toxicity or Weight Loss Observed

SBT-100: Camelid-Derived, Single Domain Antibody (sdAb)



- VHH = Single Domain Antibody (sdAb)
- Most other sdAbs do not penetrate the cell
- Small size permits deep tissue penetration
- Renal Secretion, No toxicity

Affinity Binding of SBT-100 sdAb in Biacore Assay

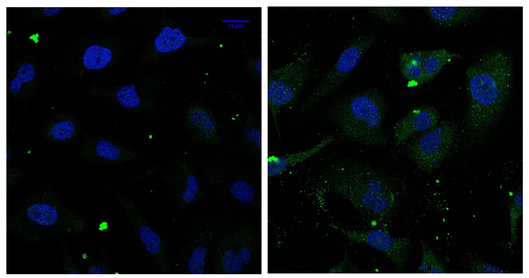
		Human KRAS Wildtype Protein	Human KRAS (G12D) Mutant
1	Anti-KRAS VHH (SBT-100)	4.20 x 10^{-9}	1.50 x 10^{-8}
2	Anti-KRAS VHH (SBT-102)	3.22 x 10^{-9}	1.48 x 10^{-7}

KD (M)

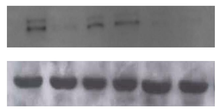
SBT-100 Binding in the Nucleus & Cytoplasm

DAPI Treated & No VHH Antibody

DAPI & SBT-100: Positive Staining in Nucleus and Cytoplasm



SBT-100 Reduces P-ERK in KRAS Mutant Cells



	Lane	P-ERK 1/2
1	MDA-MB-231 Control	0.747
2	MDA-MB-231 / SBT 100: 100 µg/ml	0.347
3	PANC-1 Control	0.502
4	PANC-1 / SBT100: 100 µg/ml	0.465
5	BxPC3 Control	0.310
6	BxPC3 / SBT100: 100 µg/ml	0.292

- MDA-MB-231 & PANC-1 cells have KRAS activating mutations.
- BxPC3 cells **do not** have KRAS mutation.

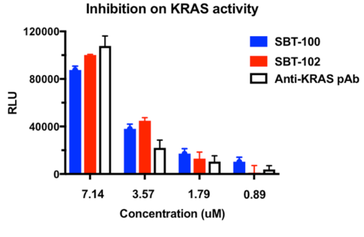
Summary: KRAS Binding & Inhibition by SBT-100

- Biophysical Data: SBT-100 binds to KRAS & KRAS(G12D) in a Biacore assay with nanomolar affinity (K_D = 10^{-8} – 10^{-9} M).
- Biochemical Data: SBT-100 inhibits KRAS GTPase activity equivalent to the polyclonal anti-KRAS antibody.
- Western Blot: SBT-100 down regulate p-ERK expression in cancer cells with activated KRAS mutations.
- MTT Assay: SBT-100 significantly inhibits cell growth of human cancers with KRAS mutations in vitro.
- In Vivo Xenograft Study: SBT-100 significantly inhibits the growth of human tumors with KRAS mutations.

Single Domain Antibodies (sdAbs)

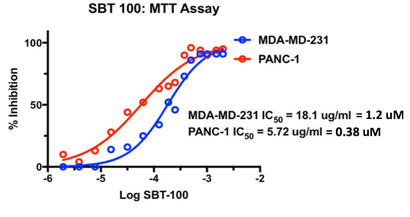
- The Single Doman Antibody (sdAb) from camelid, also known as VHH antibodies, is at the forefront of antibody research in HIV, cancer, and other cellular diseases.
- sdAbs lack light chains and are smaller and more stable than conventional antibodies, yet they possess fully functional antigen-binding capacity.
- Being the smallest fully functional antibody fragments, single domain antibodies (sdAbs) have many outstanding physical properties such as being able to be very efficiently produced and highly stable, they represent ideal building tools to create more elaborate molecules especially bi-specific antibodies.
- Bi-specific single domain antibodies, which are capable of simultaneous binding to two different targets, are considered the most promising solution to increase therapeutic activity to cancer cells.
- Due to its size (15 kDa), a single domain antibody is adept at reaching otherwise inaccessible targets that may play a crucial role in the molecular mechanisms of disease.

Assay: Inhibition of KRAS GTPase Activity



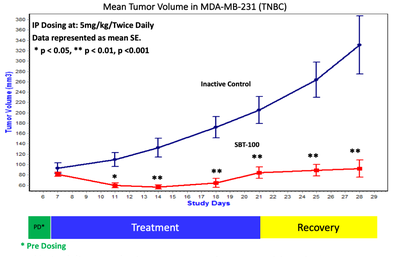
Promega assay measures GTPase activity of KRAS as it converts GTP to GDP. Background Subtracted

SBT-100 *In Vitro* Dose Response Curves



- MDA-MB-231 Cells [KRAS (G13D) Mutation]
- PANC-1 Cells [KRAS (G12D) Mutation]
- Doses response for SBT-100 in PANC-1 & MDA-MD-231 cancer cell lines *in vitro* MTT assay.

SBT-100 *In Vivo* Efficacy: TNBC



- No toxicity or weight loss observed through out the study
- Dosing for 14 days followed by 7 days of recovery and observation

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