Preclinical evaluation and biomarker identification for BNC101 anti-LGR5 mAb in K-Ras Mutant CRC and Solid Tumors

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Introduction

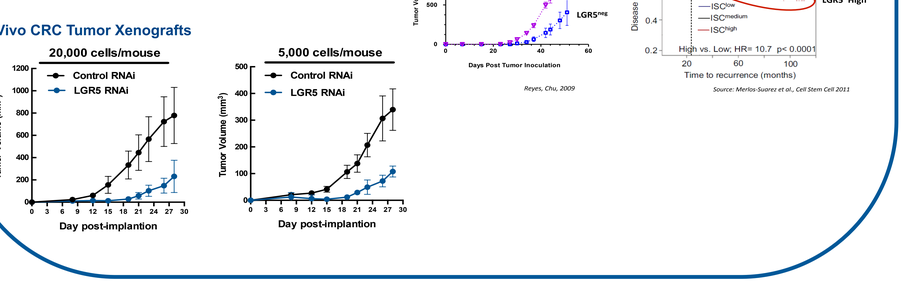
- LGR5 is a cancer stem cell receptor overexpressed in colorectal cancer (CRC), pancreatic, breast and most other solid tumors
- LGR5 is a co-receptor in the Wnt signaling pathway that focuses and enhances stem cell signaling to LGR5+ normal and cancer stem cells
- LGR5 therapeutic mAb candidates for preclinical development were selected based on biochemical characteristics, and in vitro and in vivo functional activity against cancer stem cells (CSCs) from primary patient-derived CRC xenograft (PDX)
- BNC101 is a humanized anti-LGR5 monoclonal antibody that has successfully completed IND studies for IND-filing and Phase I clinical studies in 2015

BNC101 therapeutic strategy is to delay recurrence and/or metastatic disease by preventing CSCs from re-seeding cancer following front-line surgery and standard of care chemotherapy

Background

Fig 1. LGR5 Marks Normal and CSCs in the Colon

LGR5 expression is highly specific to normal intestinal stem cells

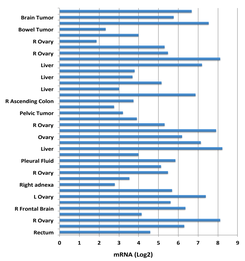


LGR5 cells are cancer stem cells at the root of colon cancer

Fig 2. LGR5 is Highly Expressed in Primary and Metastatic Cancer

Indication	Assay	% LGR5 Expression (n)	Fold-increase
Colorectal	Microarray	87% (77)	6.7
Metastatic Colon	Microarray	NA	16
Ovarian	qRT-PCR	53% (33)	>3
	Microarray	83% (36)	8.9
Pancreatic	Microarray	27% (11)	3-4
	IHC	100% (17)	2
Metastatic Breast	FACS	3-6% of cells	NA
Lung	Microarray	20% (15)	2-4
Metastatic Prostate	FACS	1% of cells	NA
Gastric	IHC	91% (32)	2.5
Esophageal	IHC/PCR	85% (60)	3.5
Liver (HCC)	qRT-PCR	53% (38)	>3
β-cat mutant HCC	qRT-PCR	100% (11)	>2

LGR5 Expression in Metastatic CRC Panel



LGR5 Expression in Origene Tumor Tissue Array

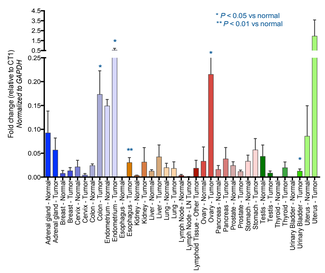


Fig 3. LGR5 RNAi Knockdown Inhibits Growth of Colorectal Tumor Lines

Soft Agar

siRNA treatment of HCT116

In Vivo CRC Tumor Xenografts

Fig 4. LGR5 Marks Highly Tumorigenic CRC Cells and Predicts Relapse in CRC Patients

Sorted LGR5+ Cells from Patient Derived Tumor

Reyes, Chu, 2009

LGR5hi ISC Gene Signature Predicts 10-fold Higher Disease Relapse in CRC Patients

Source: Merlos-Suarez et al., Cell Stem Cell 2011

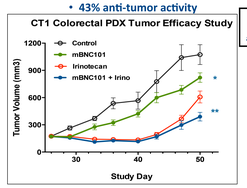
Results

Figure 5. BNC101 Targets CSCs in K-Ras Mutant CRC That Seed New Tumors

A. Colorectal Cancer Patient-Derived Xenograft Study #1:

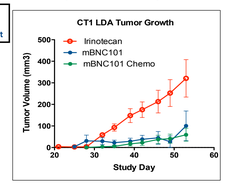
Patient CT1: Stage IV, mCRC (liver) with mutations in K-Ras, PI3K, PTEN, p53

Outsized Tumor Inhibition Activity
- ~10% Lgr5+ cells in CT1
- 43% anti-tumor activity



Re-implant tumor cells after treatment

LDA: Tumor Growth Inhibition of 30 cell Re-implant after Primary Treatment ¥

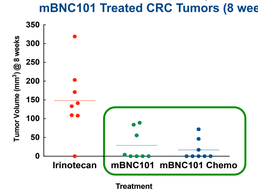


* P < 0.001 vs Control
** P < 0.001 vs Irinotecan
Initial tumor size ~ 200 mm³

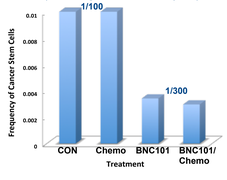
¥No additional treatment in this experiment

B. Limiting Dilution Assay (LDA) on CT1

Tumor-Free Mice After Re-Implantation of mBNC101 Treated CRC Tumors (8 weeks)



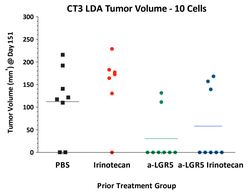
mBNC101 Reduces CSC Frequency (Limiting Dilution Analysis)

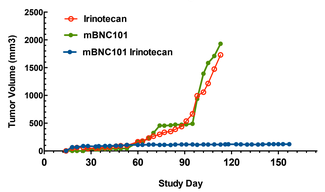


C. Colorectal Cancer Patient-Derived Xenograft Study #2:

Patient CT3: Stage IIIb CRC, with mutations in K/H-Ras, PI3K, PTEN, APC, STK11, RB1, TP53, FGFR2

mBNC101 Treatment Prevents Tumor Re-Growth in Serially Transplanted Hosts*

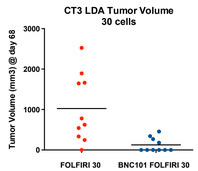


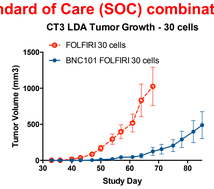


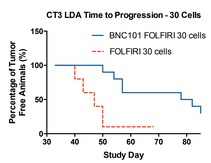
* No additional treatment in this experiment

D. Colorectal Cancer Patient-Derived Xenograft Study #3:

Patient CT3: BNC101 FOLFIRI Standard of Care (SOC) combination

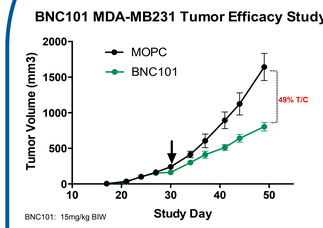


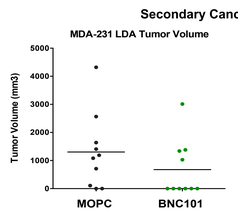




Results

Figure 6. Single Agent Activity of BNC101 in Triple-Negative Breast PDX Models





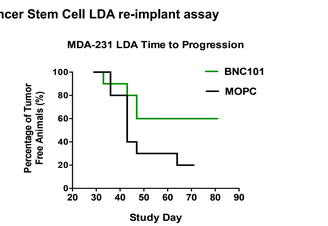
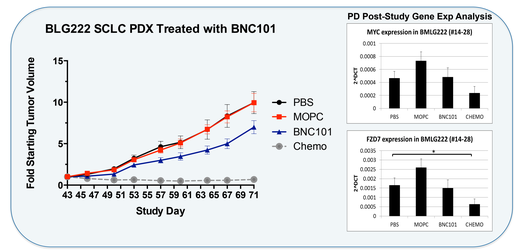


Figure 7. BNC101 Activity in Small-cell Lung Cancer (SCLC) PDX Models



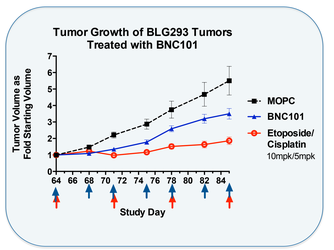
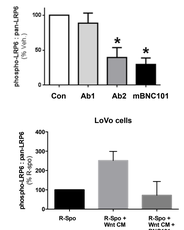
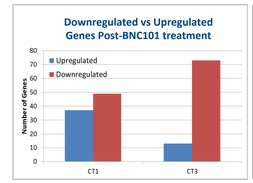


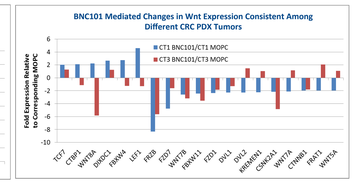
Fig 8. Modulation of Wnt proteins and target genes in CRC tumors treated with BNC101

BNC101 reduction of pLRP protein



BNC101 modulates the expression of a panel of Wnt genes





Study Design: 1) cDNA from n=10 BNC101 or MOPC treated CT1 or CT3 tumors was pooled. 2) QPCR in Wnt Plates Containing Primers for 84 genes. 3) Data Analyses

Fig 8. Average Inhibition of LRP6 phosphorylation by BNC101 (murine 18G7) without added RSPO or Wnt, and with R-spo and Wnt (CM-conditioned media) ligands

BNC101 Summary and Conclusions

- **BNC101: First-in-Class mAb targeting LGR5 CSCs, Active in: K-Ras mutant CRC, Triple-negative breast and Lung cancer Patient-derived Xenografts**
- **Targets and Reduces CSCs in vivo, Prevents Tumor Re-Growth in Re-implant Assays**
- **BNC101 combined with FOLFIRI significantly Delays Tumor Recurrence in CRC PDX re-implantation models**
- **BNC101 treatment of CRC and SCLC PDX tumors Modulates Wnt Gene Expression**

BNC101 LGR5 mAb treatment may significantly extend patient survival in CRC, TNB, SCLC and also Pancreatic Cancer

Wnt genes and LGR5+ CTCs identified as BNC101 biomarkers for upcoming Phase I trial