

BNC101 – A LGR5 specific therapeutic antibody

At the base of normal intestinal crypts, stem cells maintain the highly regenerative gut epithelium. These intestinal stem cells are well characterized for their high expression of the G-protein coupled receptor LGR5 (also known as GPR49). Together with R-spondins, (potent Wnt/ β -catenin signaling modulators) and stem cell growth factors, LGR5 forms part of a signaling cascade responsible for the regulation of cellular proliferation.

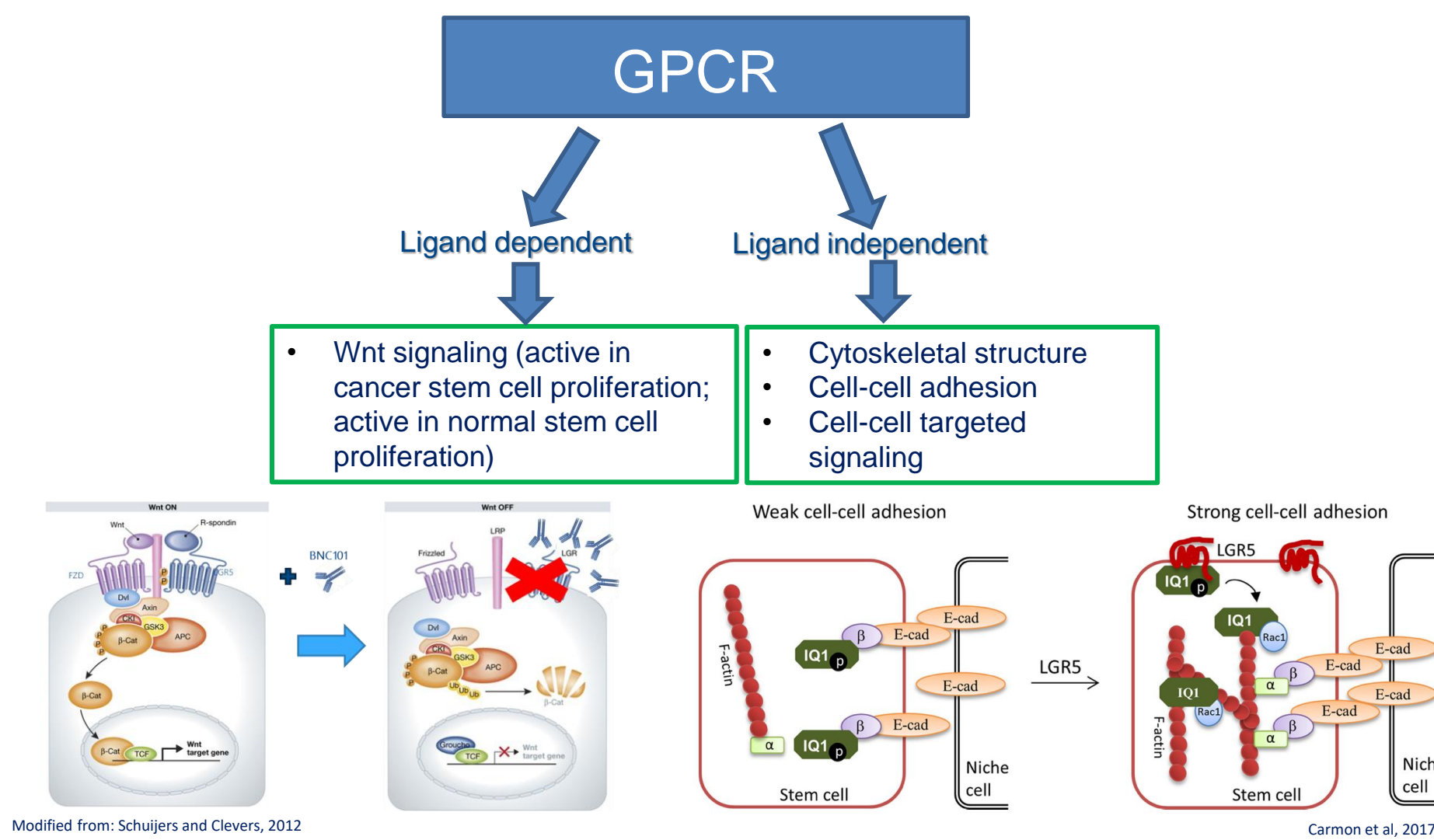
Key mutations in the APC or BRAF pathways of intestinal stem cells lead to lesions and metastatic colorectal cancer if not diagnosed and resected at an early stage. The majority of primary and metastatic tumors arising from these mutations over-express LGR5. It has been reported that metastatic colorectal cancer (CRC) patients expressing higher levels of LGR5 in tumor biopsies had increased rates of relapse.

BNC101 is a first-in-class high affinity anti-LGR5 humanized monoclonal antibody that has been shown pre-clinically to have anti-tumor activity in multiple CRC patient-derived xenografts and in limiting dilution re-implantation assays, consistent with the hypothesis that LGR5 is a functional cancer stem cell (CSC) target in CRC.

A Phase I clinical study of BNC101 in recurrent metastatic CRC patients has recently been completed.

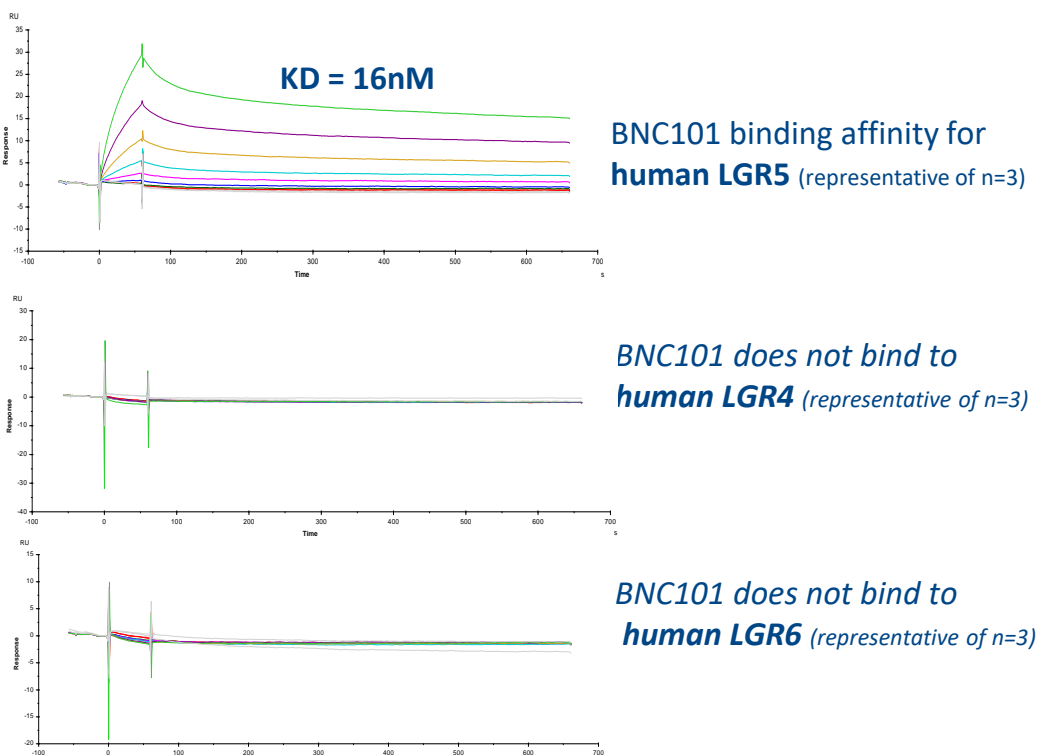
Here we explore the specificity and internalization of BNC101 and for the first time show co-localization of LGR5 with BNC101 in patients.

Two Proposed Actions of LGR5 signaling



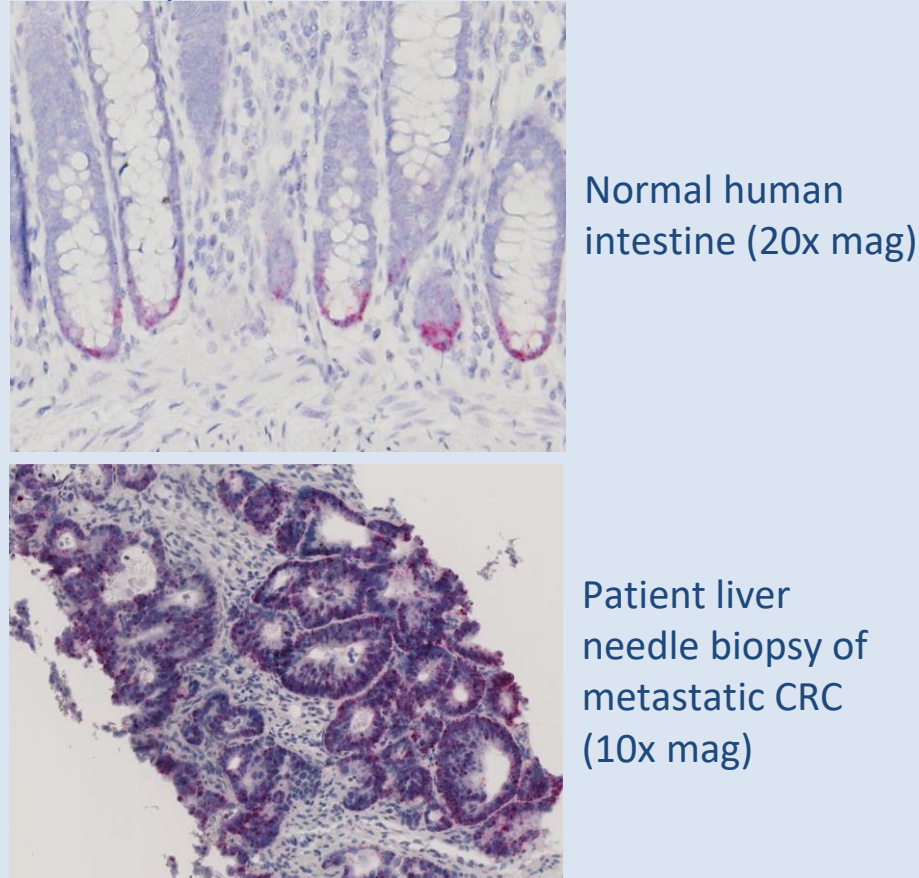
BNC101 Specificity

Biacore SPR analysis of the humanized monoclonal antibody BNC101 demonstrated a high affinity to LGR5 (KD=16nM) with no cross-reactivity to LGR4 or LGR6 receptors.



LGR5 expression

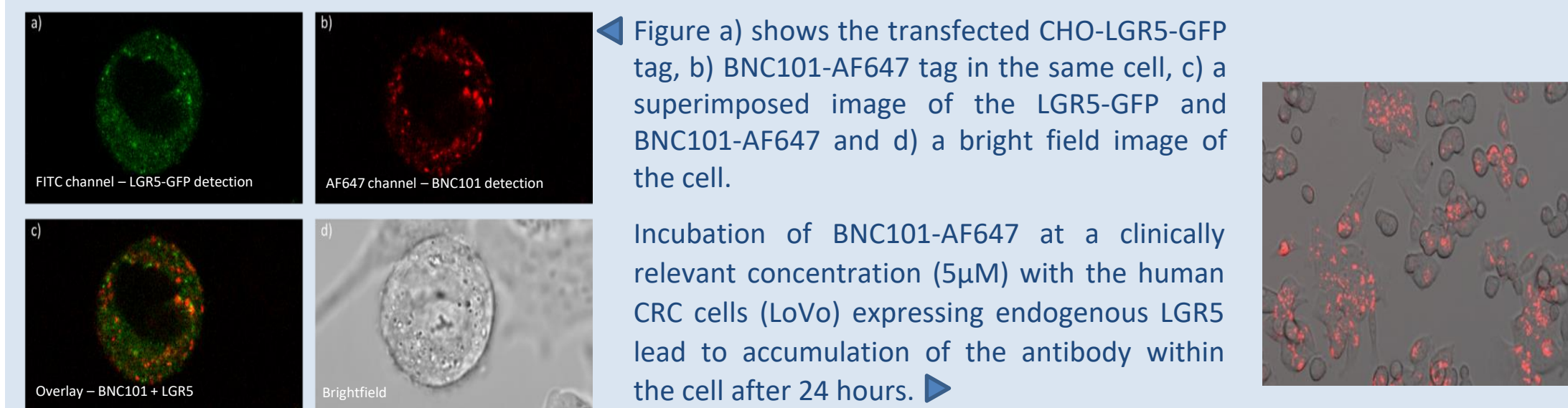
Over-expression of LGR5 in normal vs tumor cells



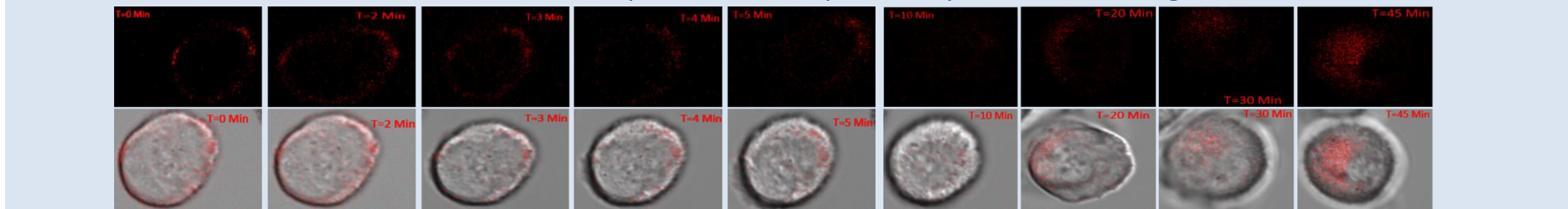
LGR5 mRNA expression was determined by RNAScope *in situ* hybridization with a human LGR5 probe.

Cellular binding and internalization of BNC101

BNC101 was conjugated to Alexa Fluor 647 (BNC101-AF647) as a tool to demonstrate binding and co-localization in cells. Initially CHO cells were transfected with DNA encoding for LGR5 with GFP (green tag). Once established, these cells were incubated with BNC101-AF647 (BNC101 with a red tag) to determine co-localization.

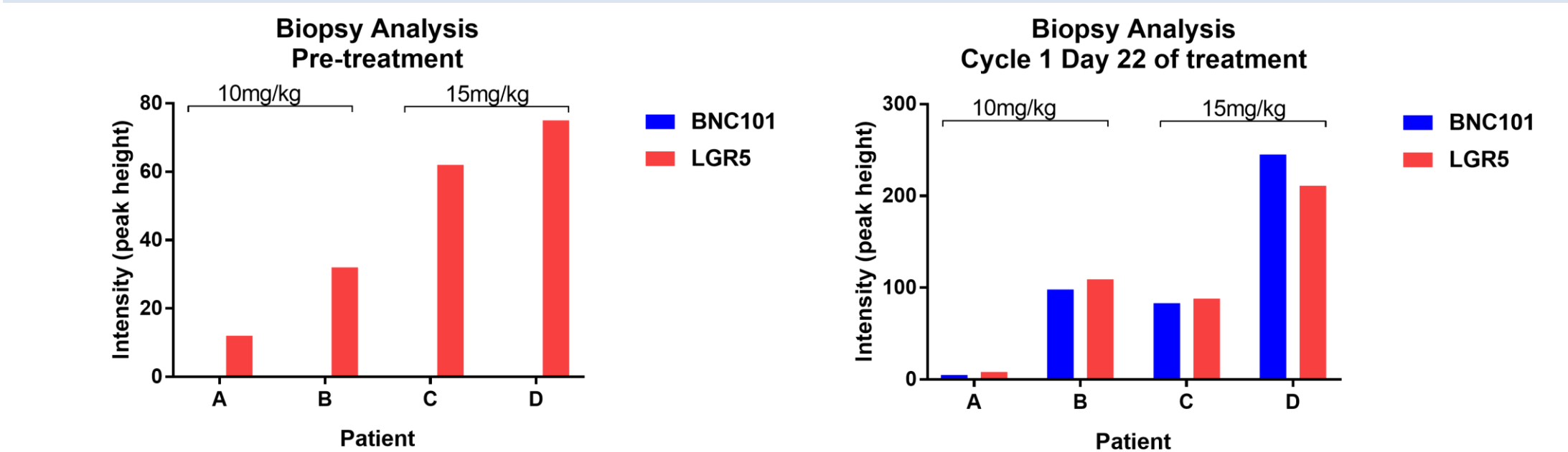


Time lapse photography in the CHO cell line clearly shows both internalization and accumulation of BNC101-AF647 over 45 minutes. At this time there is an increase in fluorescence intensity to one side of the cell, suggesting BNC101-AF647 has accumulated within the cell but seems to be compartmentalized potentially to the Trans-Golgi network.

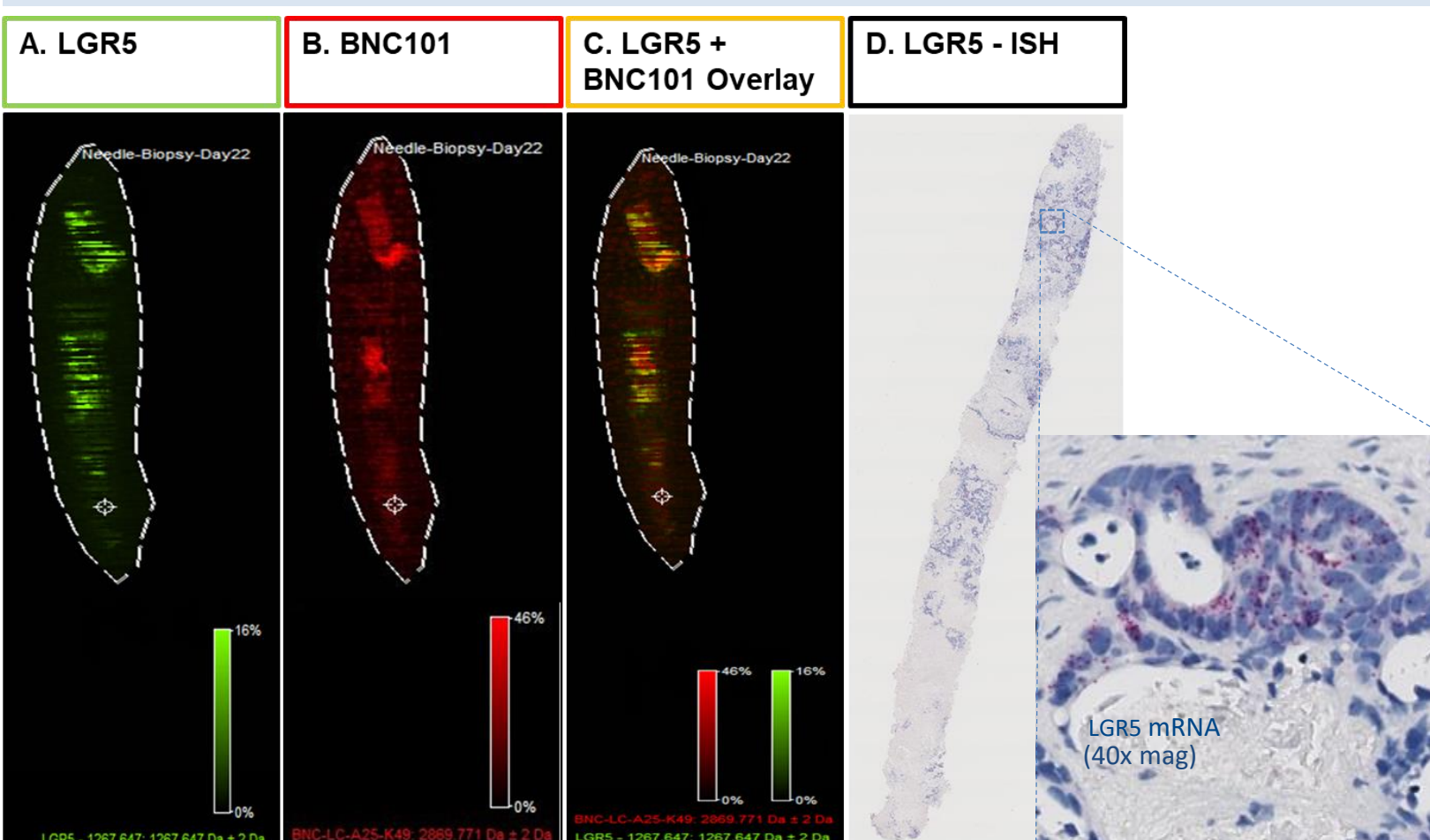


Co-localization of LGR5 and BNC101 in patients

A Phase I safety and dose escalation clinical trial of BNC101 in patients with recurrent metastatic CRC has been completed. Tumor biopsies were analyzed by LCMSMS together with Matrix Assisted Laser Desorption/Ionization mass spectrometric imaging (MALDI MSI) and we were able to demonstrate BNC101 target engagement with LGR5 for the first time in tumor biopsies following treatment.



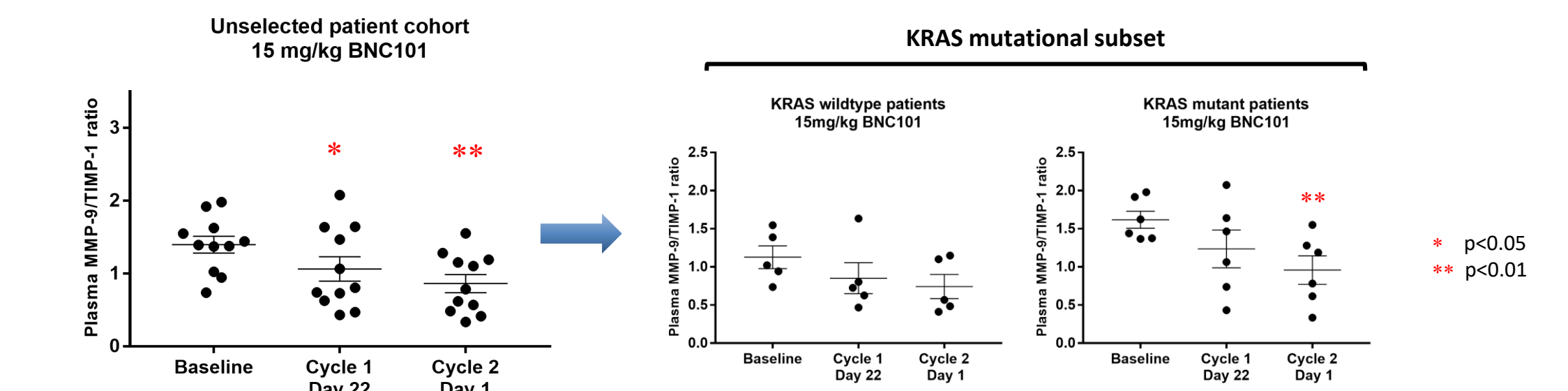
Spatial ion intensity images of mass filters that align with tryptic peptides from BNC101 Light chain and LGR5: Tryptic peptide MALDI-MSI was performed on 6 μ m thick FFPE tissue section from Patient B after 22 days of treatment with BNC101. MALDI-MSI data acquired was used to generate the ion intensity images using fleximaging software.



A. Ion intensity image for m/z 1267.647, which may correspond to a tryptic peptide from LGR5, B. Ion intensity image for m/z 2868.305, which may correspond to a tryptic peptide from BNC101 Light Chain, C. overlay of m/z signals from A. and B. All signal intensities shown have been normalized against the Total Ion signal and intensity scales for each ion are included. D. RNAScope image showing LGR5 mRNA from the same tumor needle biopsy demonstrates presence of LGR5 and tumor in similar tissue regions.

MMP-9/TIMP-1 ratio: A potential clinical PD biomarker

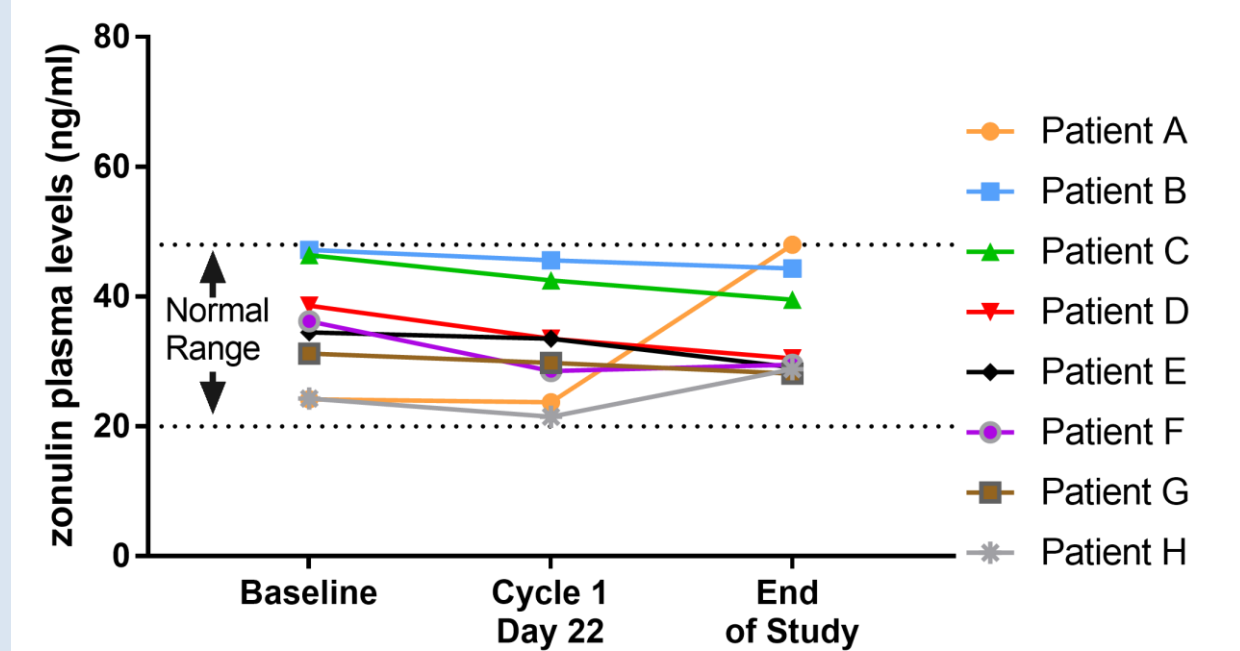
Patient plasma samples were analyzed for the soluble proteins MMP-9 and TIMP-1 by ELISA. The matrix metalloproteinase MMP-9 is known to degrade extracellular matrix (ECM), and in the tumor microenvironment (TME) is associated with increased metastatic potential and motility. The tissue inhibitor of MMPs (TIMPs) bind and inhibit enzymatically activated MMPs at a 1:1 molar stoichiometric ratio, inhibiting MMP proteolytic activity. Matrix remodeling, angiogenesis and migration are key functions and in a normal environment, increased expression of one would illustrate an increase in expression of the other. Thus reduced MMP inhibitory mechanisms or enhanced MMP expression may disrupt the MMP/TIMP ratio within the TME. The MMP-9/TIMP-1 ratio was investigated herein to determine whether an imbalance in these two proteins exists, potentially indicative of changes in the tumor microenvironment. We observed a statistically significant decrease in the ratio of plasma levels of MMP-9 and TIMP-1 which provides support for a pharmacodynamic effect following treatment with BNC101 at 15 mg/kg, and in particular in patients that expressed the KRAS mutation, this may be useful for patient selection in future clinical investigations.



BNC101 treatment is not associated with gut toxicity

Plasma zonulin levels from patients recruited in the Phase 1 clinical trial were measured by ELISA and quantitatively analyzed. As BNC101 is an antibody targeting LGR5, which is highly expressed in normal intestinal crypt stem cells, it was imperative to examine BNC101 impact on normal gut physiology. Zonulin is a clinically accepted marker of gut health and is routinely used to determine if adverse drug effects have occurred within the gut. The data suggest that no significant changes to zonulin levels outside of normal range were detected during BNC101 treatment. This result would imply that gut barrier function has not significantly deteriorated during the course of treatment.

Plasma zonulin levels in patients treated with BNC101 - 15mg/kg



First in-human evidence of BNC101 and LGR5 co-localization

- The humanized monoclonal LGR5 antibody BNC101 binds to its target with nanomolar affinity and does not have off target binding activity with LGR4 or LGR6.
- BNC101 binds to surface LGR5 of human colorectal cell lines, is internalized and accumulates within the cell.
- BNC101 has been shown for the first time in human to co-localize with LGR5 in patient metastatic CRC biopsies.** This was done using novel methodologies including LCMSMS and MALDI-MSI without the need for dosing patients with a labeled antibody.
- A potential PD biomarker has been identified with significant changes to the MMP-9/TIMP-1 ratio in patients treated with 15mg/kg BNC101. The significance observed was driven by the KRAS mutation which may aid in patient selection for future clinical trials.
- This data from the Phase I clinical trial supports further clinical evaluation of BNC101 in combination with standard of care therapies.**

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Carmon K, Gong X, Yi J, Wu L, Thomas A, et al. 2017. LGR5 receptor promotes cell-cell adhesion in stem cells and colon cancer cells via the IQGAP1-Rac1 pathway. J Biol Chem. 8;292(36):14989-15001