Targeting the LGR5 complex with BNC101 to improve checkpoint inhibitor therapy in colorectal cancer

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BNC101 - A LGR5 specific therapeutic antibody

LGR5 is a well characterised marker of intestinal stem cells found at the base of intestinal crypts and is an allosteric modulator of the Wnt / β-Catenin signalling pathway in colorectal cancer (CRC). Overexpression of LGR5 in CRC patients has been shown to be a predicative marker of higher recurrence.

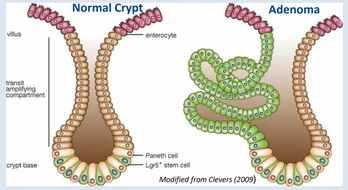
BNC101 is a first-in-class high affinity anti-LGR5 humanized monoclonal antibody that has been shown pre-clinically to have anti-tumor activity in multiple CRC patient derived xenografts and in limiting dilution re-implantation assays, consistent with the hypothesis that LGR5 is a functional cancer stem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

CSCs have been shown to have an immunosuppressive ability contributing to cancer progression.

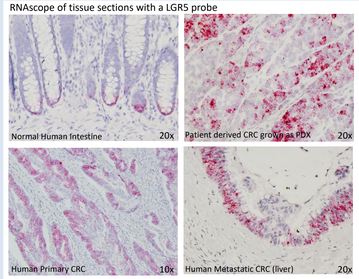
BNC101 is currently in a Phase I clinical study to treat patients with recurrent metastatic CRC.

Microsatellite instability-high (MSI-H) metastatic CRC has recently shown promising response to treatment with the checkpoint inhibitor nivolumab, an anti PD-1 inhibitor (NCT02060188). Further activity of checkpoint inhibitors in the treatment of metastatic CRCs may be achieved by targeting the immunosuppressive CSC LGR5+ve component of tumors.

Here we explore the potential synergies and benefits of targeting immunosuppressive CSCs using BNC101 in combination with the checkpoint inhibitor anti-PD-1.

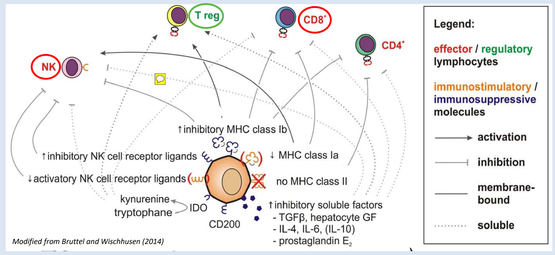


RNAscope of tissue sections with a LGR5 probe



Cancer stem cells generate an immunosuppressive TME

Latent tumors may be initiated by long-living CSCs that are known to avoid immunosurveillance. CSCs create an immunosuppressive tumor microenvironment (TME) through receptor and cytokine signalling which dampen the host anti-tumor immune response. Daughter cells however are more immunogenic and can be eliminated by normal adaptive and innate immune activity which is further activated by checkpoint inhibitors therapies.



CSC down-regulate MHC-I and lack MHC-II expression, inhibit CD8s and DC maturation and activate T-regulatory (Tregs) cells.

This leads to an alteration in tumor and draining lymph node antigen processing systems and tumor antigen presentation.

Driving a BNC101 and checkpoint inhibitor clinical combination

The clinical strategy is to use BNC101 in combination with standard-of-care (SOC) to inhibit CSC activity and/or directly eliminate cancer stem cells. As a result, BNC101 is proposed to significantly increase the duration of response and survival compared to current SOC therapies for colorectal cancer.

The combination of BNC101 with a checkpoint inhibitor may drive a therapeutic outcome whereby:

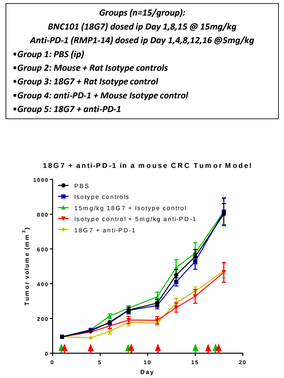
- **The immune system is activated against differentiated tumor bulk**
- **The immune-evasive residual CSCs would be reduced by LGR5 targeting**
- **The immune system locally activated by elimination of CSCs receptors and immunosuppressive cytokines**
- **Innate recruitment of Natural Killer cells to the tumor through antibody-dependent cell-mediated cytotoxicity**

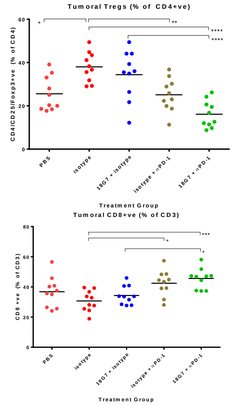
Preclinical model of anti-LGR5 in combination with anti-PD-1

The syngeneic murine MC38 colorectal cancer model was used to explore the potential synergies of BNC101 and anti-PD-1 treatments. Anti-PD-1 was the effector in terms of growth inhibition as expected from the MC38 model.

The combination of murine versions of both antibodies drove a reduction in tumoral Tregs (FoxP3+) (35%) and increase in tumoral CD8+ve cells (7%) when BNC101 and anti-PD-1 were used compared to anti-PD-1 treatment alone.

Groups (n=15/group):
BNC101 (18G7) dosed ip Day 1,8,15 @ 15mg/kg
Anti-PD-1 (RMP1-14) dosed ip Day 1,4,8,12,16 @5mg/kg
- ***Group 1: PBS (ip)***
- ***Group 2: Mouse + Rat Isotype controls***
- ***Group 3: 18G7 + Rat Isotype control***
- ***Group 4: anti-PD-1 + Mouse Isotype control***
- ***Group 5: 18G7 + anti-PD-1***





Tregs are a major immune resistance mechanism crucial for maintaining self-tolerance.

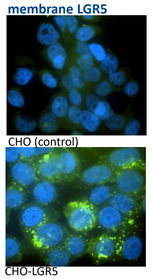
Cytotoxic T cells (CD8+ve) are a major immune effector cell.

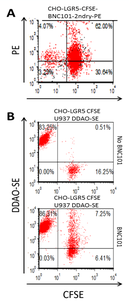
This is a significant finding and shows early efficacy supporting the rationale that CSCs create an immunosuppressive environment which can be targeted in combination for a better immunotherapeutic response.

Antibody-dependent cell-mediated cytotoxicity

Natural Killer (NK) cells use a mechanism similar to CD8+ve cells without the need for clonal T cell receptor recognition. NK cells which express Fc receptors (mostly CD16) are activated and lyse target cells in humans by the presence of the Fc fragment of surface bound antibodies, typically IgG , such as BNC101.

BNC101 binds to membrane LGR5





Strong binding of BNC101 to CHO-LGR5 cells

No interaction of monocytes and CHO-LGR5 cells without BNC101

Cross binding of monocytes to CHO-LGR5 cells in the presence of BNC101 ➡ Potential for Fc-mediated ADCC

The activation of antibody-dependent cell-mediated cytotoxicity (ADCC) was demonstrated using BNC101 treatment of cells.

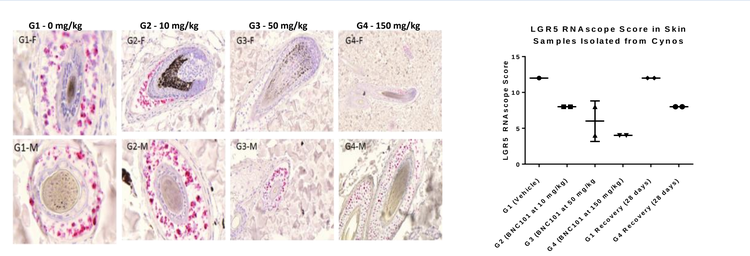
Membrane dye tracked U-937 monocytes (which express FcR) were able to specifically cross bind with CHO-LGR5 cells but only in the presence of BNC101 (an IgG1).

The recruitment of NK cells specifically to LGR5+ve cells by BNC101 binding may complement the action of checkpoint inhibitors through:

- **Direct lysis of LGR5+ve cells;**
- **Release of cell type antigen and amplification of immune activation**

LGR5 target engagement

Cynomolgus monkeys were dosed with BNC101 at 0, 10, 50, and 150 mg/kg in a GLP toxicology study. LGR5 content was assessed in hair follicles, using *in situ* hybridization technology and a human LGR5 probe. LGR5 expression in monkey skin biopsies was assessed by blinded pathologist scoring (G1 - 0 mg/kg; G2 – 10 mg/kg; G3 – 50 mg/kg; G4 – 150 mg/kg). No difference was noted between female and male monkeys (F:female; M:male).



A qualitative reduction in LGR5 mRNA is seen in Cynomolgus monkey at a clinically relevant dose range of BNC101.

Phase 1 clinical study - Exploratory biomarkers

Phase I monotherapy: determine safety, RP2D, PK, immunogenicity, target engagement, tumor response and develop LGR5+ specific biomarkers.

Standard 3 + 3 dose escalation to determine the RP2D followed by an expansion of the RP2D cohort.

Exploratory biomarker analysis is planned using both invasive and non invasive patient samples to monitor key immune events within the tumor after BNC101 treatment and validate the pre-clinical findings.

Non-Invasive sample Blood:
Experimental biomarkers including immune markers/immunophenotyping, exosome analysis, PBMC and circulating cell free DNA molecular analysis.

Invasive sample Patient tumor biopsy:
Patient Biopsy material will be analysed using the Nanostring nCounter® Profiling Panel (custom) to investigate stem cell markers, immunomodulation and Wnt / β-Catenin signalling.

Exploratory biomarker analysis of the BNC101 treated tumor and periphery will add supporting data towards the combination of BNC101 and checkpoint inhibitors.

Improving checkpoint inhibitor therapy in colorectal cancer

The combination of immune checkpoint therapies with BNC101, a LGR5/CSC targeting agent, may improve the clinical utility of each approach.

These early findings of T Regulatory cell reduction and CD8+ve cell increase within the tumor when the two therapies are used in combination support the hypothesis that targeting the CSC component reduces the immunosuppressive activity of CSCs.

This early data and further evidence expected from the Phase I clinical trial supports the clinical evaluation of BNC101 in combination with checkpoint inhibitors.

Releasing the immunosuppressive capabilities of CSC with BNC101 and targeting of the tumor bulk with checkpoint inhibitors may extend the reach of the immune system, whereby they are able to leverage greater therapeutic benefit to a larger patient population and extend duration of response.

References: Clevers, H. (2009), Searching for adult stem cells in the intestine. EMBO Mol Med, 1: 255–259. Bruttel VS, Wischhusen J. (2014), Cancer Stem Cell Immunology: Key to Understanding Tumorigenesis and Tumor Immune Escape? Frontiers in Immunology, 5:360.