BI 905711, a novel CDH17-targeting TRAILR2 agonist, effectively triggers tumor cell apoptosis and tumor regressions selectively in CDH17-positive colorectal cancer models.

Boehringer Ingelheim

Abstract # 2051

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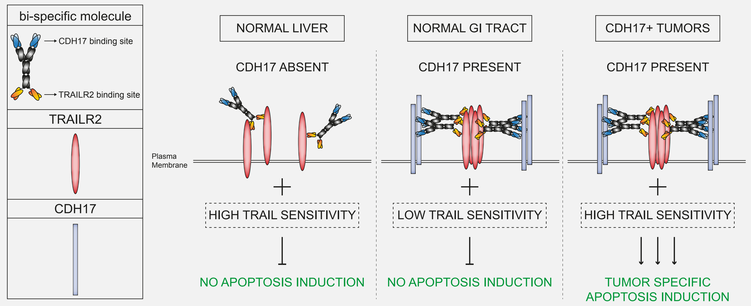
INTRODUCTION

The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) has the capability to induce apoptosis in a wide variety of tumor cells and has emerged as an important therapeutic concept for cancer treatment. To date however, TRAILR2 agonistic antibodies, designed to trigger tumor cell apoptosis, have only had limited clinical success due either to lack of efficacy or liver toxicity.

BI 905711, a tetravalent bispecific antibody targeting both TRAILR2 and CDH17, was designed to overcome the disadvantages of current TRAILR2 antibodies. CDH17-dependent clustering of TRAILR2 permits BI 905711 to selectively induce apoptosis in CDH17-expressing tumor cells. CDH17 was selected as the anchor target due to its restricted expression, in particular a lack of expression in normal liver tissue, thereby avoiding the clinical hepatotoxicity associated with TRAILR2 agonism.

Here, we report the preclinical activity of BI 905711 using colorectal cancer (CRC)-derived cell lines and patient-derived xenograft (PDX) models. We demonstrated that BI 905711 effectively triggered apoptosis in a broad range of CDH17-positive CRC tumor cells in vitro. Furthermore, BI 905711 potently initiated the apoptosis cascade as evidenced by a strong post-treatment increase of both caspase-8 and caspase-3/7. Importantly, induction of extrinsic apoptosis signaling by BI 905711 was strictly CDH17-dependent, as further demonstrated using a pair of CDH17-positive and -negative clones of the CRC cell line GP2d. When comparing the CDH17-negative clone with the parental cell line, the absence of CDH17 translated into a more than 1000-fold drop in BI 905711-dependent efficacy. Moreover, BI 905711 demonstrated single-agent tumor regressions in the GP2d colorectal cancer xenograft model. The antitumor efficacy of BI 905711 was further investigated in vivo in different CRC PDX models, showing effective tumor growth inhibition in a q14d dosing schedule.

In summary, we demonstrated that BI 905711 potently triggers the extrinsic apoptosis pathway specifically in CDH17-positive tumor cells, which translates into significant tumor growth inhibition in CRC patient-derived xenograft tumors. BI 905711 is a first-in-class molecule that shows superiority to existing TRAILR2 agonists, represents a targeted strategy for the treatment of CRC and additional CDH17-positive cancer types. Together with its potential for a favorable safety profile, these data support our plan for a phase I trial of BI 905711 in these patient populations.

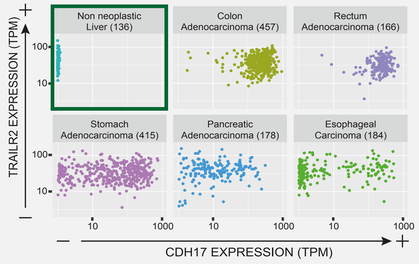


METHODS

- **FACS:** anti-CDH17 and anti-TRAILR2 BI-synthesized were used. A minimum of 10,000 events per well were collected using FACSDiva software, and exported to FlowJo version 10.1 for analysis.
- **Cell viability assay:** Serial dilutions of the corresponding antibodies were added for 24h (COLO 205) or 72h (GP2d). Cell viability was determined using the CellTiter-Glo® luminescent cell viability kit (Promega, G7571) according to the manufacturer's instructions.
- **Generation of CDH17 Knockout cells:** Cas9 genome editing constructs encoding gRNAs for CDH17 were obtained from GenScript (NJ, USA) and transfected to GP2d cells using the X-tremeGENE HP DNA transfection reagent kit (Roche, 0636236001) following manufacturer's instructions.
- ***In vivo* efficacy:** GP2d CRC cells were grafted in athymic female BomTac:NMRI-Foxn1nu purchased from Taconic. Mice were randomly distributed between the treatment and the vehicle control group (8 days after engraftment) when tumours were well established and had reached a median tumour volume of 125.5 – 137.18 mm³. BI 905711 suspended in Citrate buffer was administered intravenously.
- **IHC for c-caspase-3 and -8:** anti-cleaved caspase-3 (Asp175) (CST#9661) or anti-cleaved Caspase-8 (Asp391) (18C8) (CST#9496) were used.
- **Caspase-3/7 and 8 activity:** Cell supernatants or EDTA-plasma samples were evaluated using the Caspase-Glo 3/7 (# G8091) or Caspase-Glo 8 (G8201) Assay from Promega according to manufacturer's instructions.
- **CRC PDX *in vivo* efficacy:** Experiments were conducted at Experimental Pharmacology & Oncology Berlin-Buch GmbH (Germany).

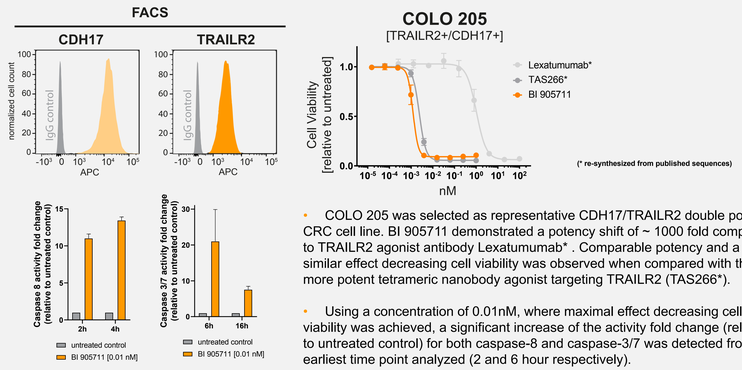
RESULTS

Figure 1: CDH17 as liver-sparing anchor to trigger TRAILR2 clustering in different oncology indications.



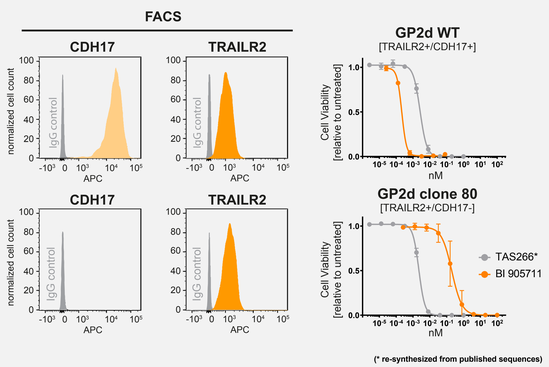
- CDH17 and TRAILR2 mRNA-expression (RNAseq) data from TCGA and GTEx.

Figure 2: Effect of BI 905711 in CDH17-positive CRC cells.



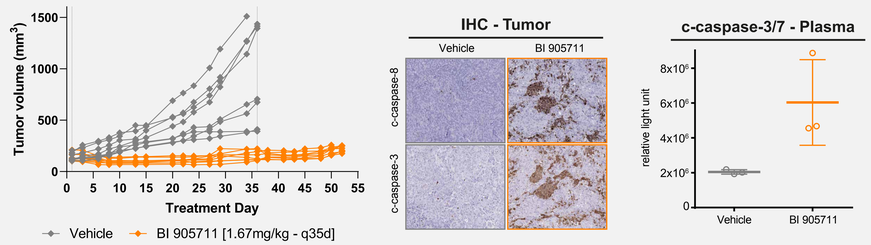
- COLO 205 was selected as representative CDH17/TRAILR2 double positive CRC cell line. BI 905711 demonstrated a potency shift of ~ 1000 fold compared to TRAILR2 agonist antibody Lexatumumab* . Comparable potency and a similar effect decreasing cell viability was observed when compared with the more potent tetrameric nanobody agonist targeting TRAILR2 (TAS266*).
- Using a concentration of 0.01nM, where maximal effect decreasing cell viability was achieved, a significant increase of the activity fold change (relative to untreated control) for both caspase-8 and caspase-3/7 was detected from the earliest time point analyzed (2 and 6 hour respectively).

Figure 3: CDH17 dependency of BI 905711 activity.



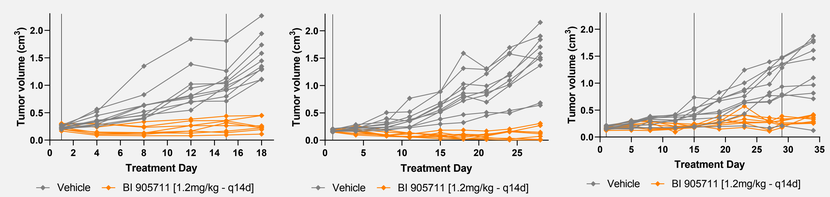
- Different CDH17-negative clones of the CRC cell line GP2d were generated using the CRISPR/Cas9 system.
- Membrane expression of both CDH17/TRAILR2 was analyzed by FACS.
- When comparing the representative CDH17-negative clone 80 with the parental cell line, the absence of CDH17 translated into a BI 905711 loss of potency ~ 1000 fold .
- The more potent tetrameric nanobody agonist targeting TRAILR2 (TAS266*) was used as reference of sensitivity to TRAILR2 agonists. Comparable potency of this CDH17-independent molecule was observed in the two cell lines.

Figure 4: BI 905711 *in vivo* efficacy and apoptosis markers modulation.



- The GP2d cell line was selected as the basis for a CRC xenograft tumor model because of a more homogeneous expression of CDH17 in vivo, comparable to that detected in human CRC tumors.
- BI 905711 demonstrated single-agent tumor regressions after single dose.
- An increased signal of apoptosis markers in tumor and plasma was seen at 24 h post dose correlating with both dose and subsequent measured response.

Figure 5: Antitumor efficacy of BI 905711 in CRC PDX models.



- BI 905711 showed effective tumor growth inhibition in a q14d dosing schedule in CRC PDX models.

SUMMARY

- **In a CDH17-dependent manner, BI 905711 triggered apoptosis in CDH17 positive CRC tumor cells *in vitro*.**
- **BI 905711 demonstrated *in vivo* efficacy after single administration in colorectal cancer xenograft models, with sustained tumor regressions up to ~30 days. Decrease in tumor size was accompanied by modulation of apoptosis markers with good correlation between tumor and plasma biomarker modulation.**
- **In CRC patient-derived xenograft tumors, BI 905711 also demonstrated a significant tumor growth inhibition.**

CONCLUSIONS

- **BI 905711 is a first-in-class molecule that shows superiority to existing TRAILR2 agonists and, together with its potential for a favorable safety profile, it represents a targeted strategy for the treatment of CRC and additional CDH17-positive cancer types.**
- **BI 905711 is scheduled to start phase I clinical trials in Q2/19.**

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