BI-907828: a novel, potent MDM2-p53 antagonist, acts synergistically in a triple combination with anti-PD-1 and anti-LAG-3 antibodies in syngeneic mouse models of cancer


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**Abstract**

Inhibition of the protein-protein interaction between the tumor protein p53 (TP53) and its key negative regulator MDM2 is a new therapeutic concept for cancer therapy. These MDM2-p53 antagonists are designed to restore p53 activity in TP53 wild-type tumors. Several of these inhibitors are currently being evaluated in early clinical development. 1

BI-907828 is a novel and potent MDM2-p53 antagonist with good oral bioavailability that is due to its reliable, dose-linear PK profile for high-dose intermittent dose schedules. BI-907828 has demonstrated efficacy in human tumor xenograft models at daily low oral doses as well as intermittent high-dose schedules. In syngeneic mouse models of cancer, BI-907828, apart from its direct tumor-targeting activity, also has an immunomodulatory property shown to contribute to efficacy. In a TP53 wild-type Colon-26 model, single-agent BI-907828 induced anti-tumor immunological memory that could be enhanced by a combination with an anti-PD-1 checkpoint inhibitor resulted in synergistic efficacy. Further, BI-907828 showed antitumor efficacy in a TP53 mutant model (MC-38) in immune-competent mice (AACR 2018, abstract 4868).

Here we present data for the triple combination of BI-907828 with antibodies targeting the immune checkpoints PD-1 and LAG-3 in syngeneic mouse models of cancer.

**Methods**

In vitro efficacy studies in syngeneic mouse models of cancer

- Colon-26 or B16-F10 cells (5x10^6, Matrigel) were injected s.c. either into BALB/c, C57BL/6 or SCID mice. 
- Mice were injected with control antibody (clone 2A3, BioXCell; 10 mg/kg), (p, qR04) plus NanomAb (vehicle), anti-PD-1 (clone RMP1-14), anti-CD8 (clone 253-774), BioXCell; 10 mg/kg), (p, qR04) plus anti-LAG-3 (clone C11B7), BioXCell; 10 mg/kg, (p, qR04), BI-907828 (10 mg/kg, p.o., q5d), or combinations thereof. Treatment was started on day 1 or 3 post cell injection. Tumor volume was measured 3 times per week and tumor regressions (+, responses, R) were defined by a relative tumor volume of ≤1 at the end of the study.

T cell depletion study in Colon-26 syngeneic mouse tumor model

BALB/c mice were randomized on day 3. Depleting antibodies or control antibodies (clone 2A3, BioXCell; 10 mg/kg) were injected i.p. at a dose of 250 μg/mouse, with 3 loading doses on days 1, 3, and 5, followed by continued administration of 1.4 mg/mouse 3 times a week. On day 7, tumors were harvested and evaluated using the 2-color flow cytometry for Colon-26 tumor infiltrating T cells. The T cell subtypes were either CD4 and CD8, CD4 or CD8.

FACS analysis of anti-tumor immune response in Colon-26 tumors

- Subcutaneous tumors were harvested at day 15 or 17 of treatment. i.e. at tumor stasis or regression endpoint. Various triple combination treatment (BI-907828, anti-PD-1, anti-LAG-3). Single cell suspensions from tumors were prepared using dissection kits from Miltenyi Biotec. Together with Life!MARCS (Resenstock) Data were collected using the BD FACSdius software (BD Biosciences). Definition of leukocyte cell populations and determination of surface marker expression was performed using the flow cytometry analysis software FlowJo (Tree Star Inc).

**Results**

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**Conclusions**

- BI-907828 is a novel, potent MDM2-p53 antagonist with optimized drug-like properties that is suitable for high-dose intermittent oral dose schedule.
- Immune modulation contributes to efficacy of BI 907828 in a TP53 wild-type Colon-26 syngeneic mouse model of cancer.
- Increased efficacy is observed in immune-competent BALB/c mice vs. immune-deficient SCID mice.
- BI 907828 acts synergistically in a triple combination with checkpoint inhibitors in TP53 wild-type Colon-26 syngeneic mouse models of cancer.
- In the Colon-26 and B16-F10 models, the triple combination of BI-907828 with antibodies against mouse PD-1 and LAG-3 shows high response rates of 90-95%, with tumor regressions observed for even very large tumors. Moreover, efficacy of the triple combination is superior to single agent and all dual combinations.
- An antibody-mediated depletion study suggests a contribution of CD8 T cells but not of CD4 T cells to full efficacy (i.e. tumor regressions) with the triple combination.
- FACS analysis of tumors isolated from Colon-26 tumor-bearing mice indicates that treatment with the triple combination leads to expansion of tumor-infiltrating CD8 T cells and a reduction in tumor-infiltrating regulatory T cells.

**References**

1 Revising the guardian of the genome: small molecule activators of p53. Pharmacology & Therapeutics 2017, vol 170, pp. 191-204

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