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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INTRODUCTION

Inhibition of the protein-protein interaction between the tumor protein p53 (TP53) and its key negative regulator MDM2 is a new therapeutic approach 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 - suitable for high-dose intermittent dose schedules. BI-907828 has demonstrated efficacy in human tumor xenograft models at daily low oral doses as well as in intermittent high-dose schedules.

In syngeneic mouse models of cancer, BI-907828, apart from its direct tumor-targeting activity, also has an immunomodulatory activity shown to contribute to efficacy. In a TP53 wild-type Colon-26 model, single-agent BI-907828 induced anti-tumor immunological memory that could contribute to a durable effect in a dual combination with an anti-PD-1 checkpoint inhibitor resulting in synergistic efficacy.

Recent BI-907828 studies showed anti-tumor efficacy in a TP53 mutant model (MC-38) in immune-competent mice (AACR 2018, abstract 4866). Here we present data for the triple combination of BI-907828 with antibodies targeting the immune checkpoints PD-1 and LAG-3 in PD-1 and LAG-3-deficient mouse models of cancer.

METHODS

In vivo 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. Mouse control antibodies (clone 2A3, BioXCell; 10 mg/kg, i.p., p.o., qd) plus Nantase (s.c.), anti-PD-1 (clone RMP1-14, BioXCell; 10 mg/kg, i.p., qd), anti-LAG-3 (clone C9B7W; BioXCell; 10 mg/kg, i.p., qd) were administered in the different groups. Control groups included only depleting antibodies (clone GK1.5, BioXCell), a CD4- (clone 2.43, BioXCell), CD4- and CD8-T cell depleting antibodies targeting the immune checkpoints PD-1 and LAG-3.

• Colon-26 or B16-F10 cells (5x10^6, Matrigel) were injected s.c. into BALB/c, C57BL/6 or SCID mice. Mouse control antibodies (clone 2A3, BioXCell; 10 mg/kg, i.p., qd). Anti-LAG-3 (clone C9B7W; BioXCell; 10 mg/kg, i.p.; clone GK1.5, BioXCell; 10 mg/kg, i.p.) were administered in the different groups. Control groups included only depleting antibodies (clone GK1.5, BioXCell), a CD4- (clone 2.43, BioXCell), CD4- and CD8-T cell depleting antibodies targeting the immune checkpoints PD-1 and LAG-3.

• Colon-26 or B16-F10 cells (5x10^6, Matrigel) were injected s.c. into BALB/c mice. Either a CD4- (clone GK1.5, BioXCell), a CD8- (clone 2A3, BioCell) and CD4-T cell depleting antibodies or an isotype control antibody (clone 17-1D4, BioCell) were administered i.p. at a dose of 250 μg/mouse, with 3 loading doses on day 1, 3 and 5, followed by continued administration of the last loading dose on day 10. On day 4 after tumor injection, a fourth dose was administered s.c. with Colon-26 tumor cells (5x10^6, Matrigel) on day 7 of the study. Treatment start was 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 0. Depleting antibodies, i.e. either a CD4- (clone GK1.5, BioXCell), a CD8- (clone 2A3, BioCell) and CD4-T cell depleting antibodies or an isotype control antibody (clone 17-1D4, BioCell), were administered i.p. at a dose of 250 μg/mouse, with 3 loading doses on day 1, 3 and 5, followed by continued administration of the last loading dose on day 10. On day 4 after tumor injection, a fourth dose was administered s.c. with Colon-26 tumor cells (5x10^6, Matrigel) on day 7 of the study. Treatment start was either with Nantase (s.c.) or control antibodies targeting PD-1 and LAG-3, both at 10 mg/kg, i.p.; clone GK1.5, BioXCell) were administered on day 4 and 7.

FACS analysis of immunological changes in s.c. Colon-26 tumors

Subcutaneous tumors were harvested at day 15 or 17 of treatment, i.e. at tumor stasis or regression, respectively. T cell depletion study in Colon-26 tumor-bearing mice was evaluated by flow cytometry after 48 h of treatment with the respective antibody. Tumor cells, harvested from tumors, were fixed in 4% paraformaldehyde in PBS supplemented with 0.1% sodium azide (pH 7.4) and kept at 4°C overnight. The cell pellets were permeabilized with 5% BSA in PBS containing 0.1% sodium azide and 0.1% Tween-20 (Pierce). FACS data were collected using the BD FACS flow cytometry device and analyzed using FlowJo software. Definition of leukocyte cell populations and determination of surface marker expression was performed using the flow cytometry analysis software FlowJo (Tree Star Inc).

RESULTS

Figure 1 Immune modulation contributes to efficacy of BI-907828

Figure 2 Triple combination of BI-907828 with anti-PD-1 /anti-LAG-3 shows superior efficacy in TP53 wild-type syngeneic models in immune-competent mice

Figure 3 CD8 T cells but not CD4 T cells are required for efficacy of the triple combination of BI-907828 with anti-PD-1/anti-LAG-3

Figure 4 BI-907828 and/or anti-LAG-3 treatment leads to a decrease in the percentage of tumor-infiltrating regulatory T cells

SUMMARY

BI-907828 is a novel, potent MDM2-p53 antagonist that shows synergistic efficacy in a triple combination with antibodies targeting the immune checkpoints PD-1 and LAG-3 in syngeneic mouse models of cancer.

Increased immune modulation contributes to efficacy of BI 907828 in a TP53 wild-type Colon-26 syngeneic model 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 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 50-90%, 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 intra-tumoral regulatory T cells.

REFERENCES


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ACKNOWLEDGMENTS

We would like to thank all technical support staff at Boehringer Ingelheim RCV GmbH & Co KGL who helped in generating the data.