A Potent Macrophage Switching Drug D-4559 Reduces Tumor Growth in a Hepatocellular Carcinoma Mouse Model

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Introduction

Hepatocellular carcinoma (HCC) is characterized by infiltration of macrophage and immature myeloid cell and dysregulated production of cytokines. Macrophages are critical mediators of tissue homeostasis and phagocytic cells of the innate immune system. They respond to the tumor microenvironment with either a pro-inflammatory or an anti-inflammatory phenotype. In early cancer stage, the dominant phenotype is the anti-inflammatory, tumor promoting M2 macrophages as opposed to the pro-inflammatory phenotype M1 macrophages (1). Tumor-associated macrophages (TAMs) are abundant in the HCC microenvironment and exhibit an immunosuppressive M2 phenotype leading to HCC progression (2). Considering TAMs have the potential ability of repolarization to M1 type macrophages to favor tumor regression (3, 4), targeting and remodeling TAM polarization is as an important therapeutic strategy in HCC (5, 6). In HCC, TAMs are also associated with sorafenib resistance, a multi-kinase inhibitor that inhibits protein kinases, including VEGFR and PDGFR (7).

D-4559 is a new class of tyrosine kinase inhibitors referred to as Dendranibs, which are metabolically stable and secreted intact through the kidneys. D-4559 is designed using the sorafenib backbone to selectively enable tyrosine kinase inhibition in TAMs leading to a functional reprogramming of TAMs toward a pro-inflammatory activated phenotype. In this study, we evaluated the effect of D-4559 on M1 reprogramming in TAMs and the anti-tumor efficacy of D-4559 in a murine HCC model, Hepa 1-6.

Methods and Materials

D-4559 was designed with a molar ratio of Sorafenib:D-4559 2:1, favoring Sorafenib free drug. In vivo efficacy of D-4559 was examined in the subcutaneous Hepa 1-6 tumor model in C57BL/6 mice. Animals (n=15/group) were treated with D-4559 (i.p., 200 mg/kg daily) and free drug Sorafenib (p.o., 40 mg/kg daily) as a positive control for 4 weeks. The treatment started when the mean tumor size reached approximately 200 mm^3, then the animals were randomly allocated into study groups. The day of randomization and treatment was denoted as day 0. Tumor sizes were measured for 27 days. At day 16, tumors were collected for M1/M2 macrophage evaluation by flow cytometry as the gating described in Figure 2. Plasma cytokine biomarkers were confirmed with Meso Scale Discovery (MSD) Cytokine Multiplex Assay using mouse plasma at days 16 and 27.

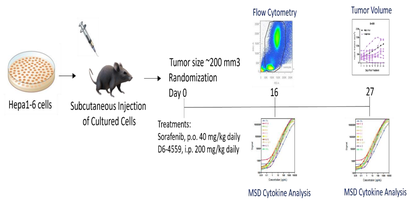

Figure 1. Study schematic

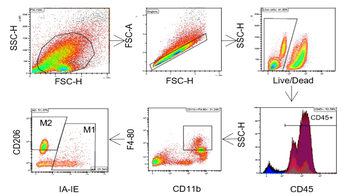

Figure 2 . Flow cytometry gating strategy

Results

Hepa 1-6 tumor growth

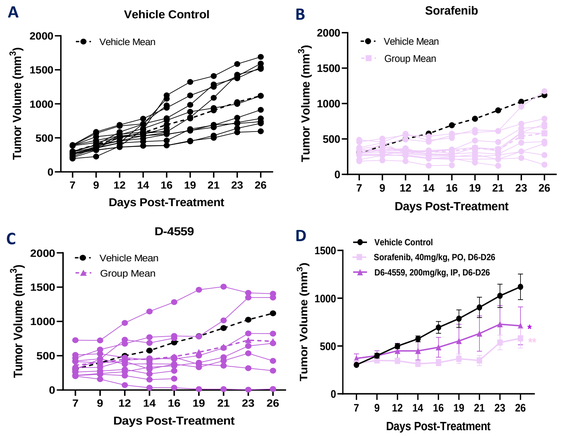

Figure 3. Hepa 1-6 tumor response to D-4559 treatment. Individual (A-C) and mean (D) tumor volumes (mm^3). Mice were subcutaneously injected with Hepa 1-6 cells and received D-4559, sorafenib or vehicle alone for 27 days. Decreased tumor size was measured in D-4559 treated group. n=15/group, Dunnett's multiple comparison test p-value *<0.05, **<0.01

D-4559 significantly reduced Hepa 1-6 tumor growth. D-4559 and Sorafenib efficacy were similar with 40% of mice with a tumor volume less than 500 mm^3 after 27 days of treatment (p=0.01).

Tumor immunoprofiling

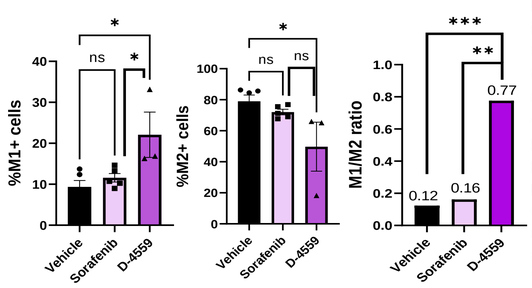

Figure 4. M1 phenotype induction in response to D-4559 treatment. At day 16 of the treatment, tumors were collected for flow cytometry. D-4559 treatment switched TAMs polarization from M2 to M1 phenotype and increased M1 phenotype by 6.4 folds compared to vehicle control. n=5/group. One-way ANOVA and Unpaired t-test, p value *<0.05, **<0.01, ***<0.001.

At day 16, D6-4559 significantly induced M1 macrophage phenotype in Hepa 1-6 tumors compared to Sorafenib, increased M1/M2 ratio by inducing M1 macrophage infiltration and reprogramming of TAMs into M1 macrophages compared to vehicle control and Sorafenib treatment groups.

Cytokine profiling

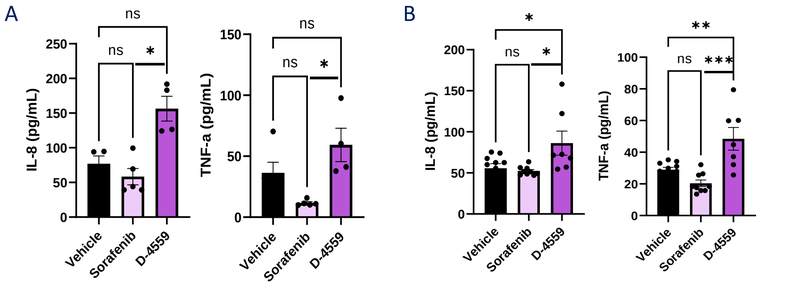

Figure 5. Changes in plasma cytokine levels after D-4559 treatment. After 16 days (A), after 27 days (B). Increased pro-inflammatory cytokines (IL-8 and TNF-a) were detected after D-4559 treatment on both days 16 and 27 by MSD using electrochemiluminescence. Mixed-effects analysis and Unpaired t-test, p-value *<0.05, **<0.01, ***<0.001.

D-4559 significantly increased IL-8 and TNF-a cytokines in Hepa 1-6 tumor microenvironment at days 16 and 27, which is an indication of a M1 signature correlated with a pro-inflammatory phenotype. Increased levels of IL-8 and TNF-a have also been correlated with better survival in HCC patients (8).

Conclusions

TAMs, major component of tumor microenvironments, are mainly polarized into an anti-inflammatory M2 phenotype related to poor HCC prognosis and an aggressive tumor growth. Modulating immunosuppressive function of TAMs would provide a therapeutic approach for HCC patients.

Dendranib D-4559 a potent macrophage switching nanomedicine technology significantly switched TAMs polarization from M2 to M1 and induced pro-inflammatory cytokine resulting in a significant tumor growth reduction in the Hepa1-6 tumor model. D-4559 can promote an immunopermissive microenvironment transition thus limit the HCC tumor growth.

This preclinical study suggests that Dendranib D-4559 can be developed as a potent agent that can be used systemically for the treatment of HCC and can advocate the development of new trials for combination therapies to improve anti-tumor immune response.

Disclosures

- Ashvattha Therapeutics funded the research.
- Alters Bioscience Consulting was contracted by Ashvattha Therapeutics to provide input on the study designs.
- Crown Bioscience was contracted by Ashvattha Therapeutics to perform the animal studies.

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