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HFB9-2, a novel Galectin-9 neutralizing antibody to reverse immune suppression in the tumor microenvironment

Germain Margall[1], Yun-Yueh Lu[1], Muriel David[1], Rachel Pacherie[1], Julie Prigent[1], Dean Lee[1], Laureen Bardouillet[1], Laetitia Claer[1], He Zhou[1], Andreas Raue[1], Roshan Kumar[1], Nicola Beltraminelli[1], Yuqiang Shen[1], Mingjie Chen[1], Jia Wu[1], Mingfang Feng[1], Angelo Paci[2a], Julia Delahousse[2a], Véronique Saada[2a], Stéphane de Botton[2b], Pierre Busson[2c], Stephanie Beq[1], Francisco Adrian[1], Liang Schweizer[1]

Contact information : g.margallducos@hifibio.com

1. HiFiBiO Therapeutics 2a. Department of Biopathology, b. Department of Hematology, c. UMR 9018 METSY, CNRS, Gustave Roussy and Université Paris-Saclay, Villejuif, France

SUMMARY

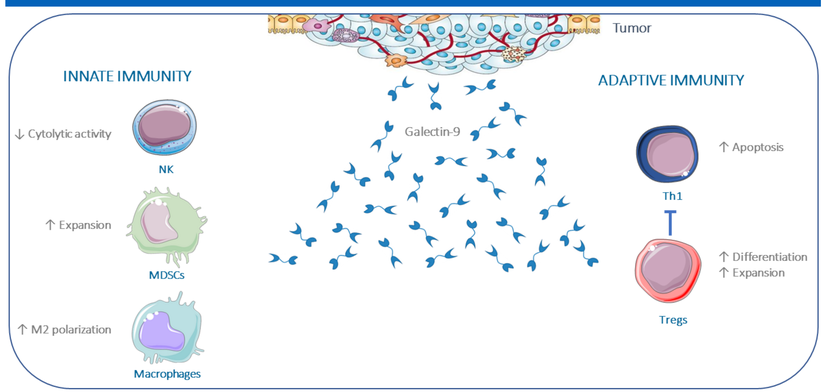
Although monoclonal antibodies targeting immune checkpoints have demonstrated clinical success in a range of tumor types, sustained responses are only observed in a fraction of patients due to primary or secondary resistance to treatment. Recent evidence has implicated the pleiotropic immunosuppressive modulator Galactoside-binding lectin Galectin 9 (Gal-9) as a key factor present in the tumor microenvironment that renders tumors resistant to current immunotherapies. High Gal-9 expression has been reported in different types of cancers including hematological malignancies such as Acute Myeloid Leukemia (AML) and Acute Lymphocytic Leukemia (ALL), and multiple solid tumors. We hypothesize that targeting Gal-9 may represent a valuable strategy to reduce immunosuppression and improve clinical response in selected cancer patients.

Gal-9 has been reported to play a dual role in AML as both a self-renewal factor for leukemic stem cells and a suppressor of anti-cancer immunity. Analysis of AML patient serum samples demonstrated that Gal-9 expression was significantly higher than in healthy controls and that Gal-9 levels dropped at complete remission. Higher levels of Gal-9 were found in French-America-British (FAB) type M4 and M5 AML samples, and the lowest levels were observed in M3 patient samples.

We present a humanized monoclonal antibody, HFB9-2, that specifically binds to human Gal-9 with sub-nanomolar affinity, recognizes recombinant Gal-9 and Gal-9 produced by human tumor cells, and is cross-reactive with mouse and monkey Gal-9 orthologs. HFB9-2 blocks the interaction of Gal-9 with its receptors TIM3 and CD44 in a dose-dependent manner. These two receptors have been described to mediate Gal-9-immunosuppressive signals in effector and regulatory T cells. Treatment of human PBMCs from healthy donors with HFB9-2 prevents Gal-9-induced Th1 cell apoptosis and suppresses the expansion of regulatory T cells induced by Gal-9. Moreover, HFB9-2 has a favorable developability profile, demonstrating stability for 30 days at 40°C, as well as for several hours at low pH, and following several freeze-thaw cycles. High plasma exposures following a single dose administration to mice were observed. HFB9-2 exhibits significant anti-tumor efficacy in the WEHI-164 syngeneic mouse model as a single agent or in combination with anti-mouse PD-1 antibody. Further analysis of the response to HFB9-2 treatment of PBMCs from AML patients is currently ongoing to guide the selection of patients most likely to benefit from HFB9-2 treatment in the clinic.

Altogether, the data presented here provide evidence that neutralization of Gal-9 with HFB9-2 blocks key immunosuppressive mechanisms known to favor cancer progression and to limit the efficacy of current immunotherapies, and position HFB9-2 as a drug candidate for clinical evaluation in AML and other indications.

INTRODUCTION



Gal-9 belongs to the tandem-repeat subfamily of galectins, which contain two different carbohydrate recognition domains (CRDs) separated by a flexible linker. Gal-9 serves as a multifaceted player in adaptive and innate immunity, implicated in several aspects of cancer progression. The most prominent effects reported for Gal-9 are the induction of apoptosis in subsets of differentiated T-cells, particularly in CD4+ T-helper 1 (Th1) cells, and a stimulatory effect on expansion of regulatory T-cells (Tregs), as well as myeloid-derived suppressor cells (MDSCs). In addition, Gal-9 is capable of impairing the cytolytic activity of natural killer (NK) cells. Gal-9 has been also shown to mediate myeloid cell differentiation toward an M2 macrophage phenotype, which exhibits potent pro-tumor activities. These findings define multiple immunosuppressive activities of Gal-9 that promote tumor escape from immune surveillance.

RESULTS

1. Gal-9 Is Broadly Expressed in Tumor Infiltrating Immune Cells

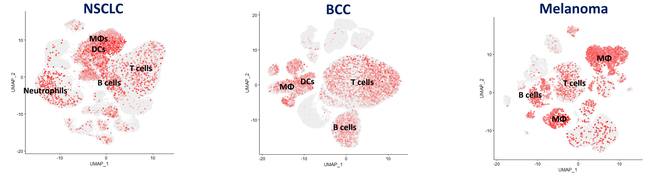
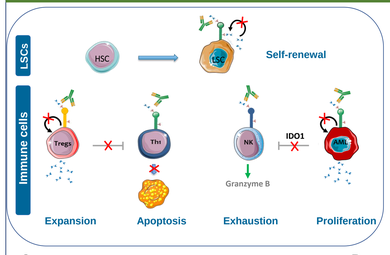


Figure 1. Single-cell RNA-seq analysis of Gal-9 expression in multiple solid tumors, including non-small cell lung cancer (NSCLS), basal cell carcinoma (BCC), and melanoma. Single-cell RNA-seq analysis demonstrated that Gal-9 is expressed broadly across tumor infiltrating immune cells such as macrophages, B cells, DCs, and T cells.

2. High Levels of Gal-9 Are Circulating in AML Patients



Gal-9 has been recently shown to impair the immunological activities of cytotoxic T cells and natural killer (NK) cells in AML, thus facilitating AML cells to escape immune attack. Moreover, a TIM-3/Gal-9 autocrine stimulatory loop has been described to regulate self-renewal of human myeloid leukemia stem cells (LSCs) and to promote leukemic progression. We have analyzed AML patient serum samples and demonstrated that Gal-9 levels are significantly higher than in healthy controls and drop at complete remission.

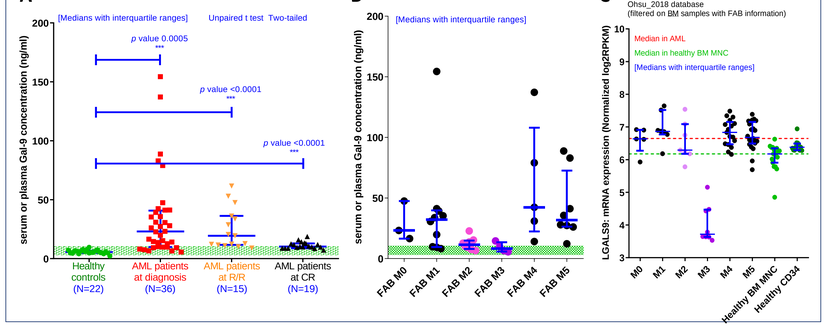


Figure 2. Translational analysis of Gal-9 levels in AML patients (A) Concentration of Gal-9 was measured in sera/plasma samples from 70 patients with AML and 22 healthy donors. Compared to the control group, the median level of Gal-9 was significantly higher in AML patients. In addition, patients in complete AML remission exhibited lower Gal-9 levels as compared to those at the time of diagnosis, but still higher than in healthy controls. (B) Higher levels of Gal-9 were found in FAB type M1, M4 and M5 AML samples, and the lowest levels were observed in M3 patient samples. (C) Serum levels of Gal-9 are in agreement with RNA-seq data that indicate lower Gal-9 mRNA level in M3 samples as compared to other AML subtypes.

3. HFB9-2 Binding Affinity and Cross-reactivity

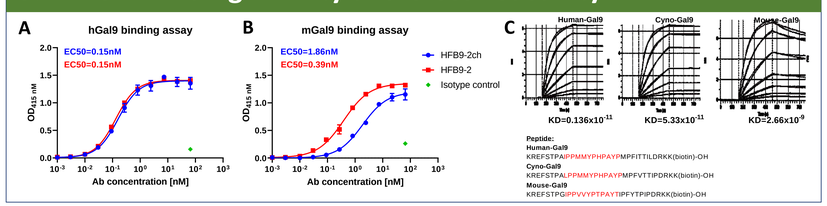


Figure 3. Binding affinity and cross-reactivity of HFB9-2, a humanized variant derived from chimeric antibody HFB9-2ch. (A) HFB9-2 binds to recombinant human Gal9 with an EC50 value of 0.15nM determined by ELISA . (B) HFB9-2 binds to recombinant murine Gal9 with an EC50 value of 0.39nM determined by ELISA (C) HFB9-2 KD values for hGal9, cynoGal9, and mGal9 determined by Octet are $0.136x10^{-11}$ M, $5.33x10^{-11}$ M, and $2.66x10^{-9}$ M, respectively.

RESULTS (cont.)

4. HFB9-2 Effects on Gal-9-Induced Tumor Escape Mechanisms

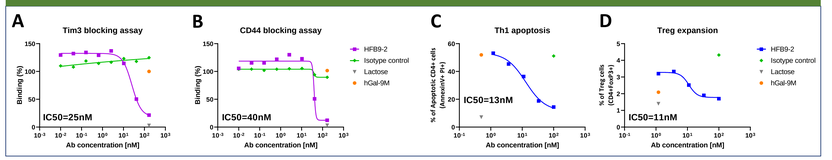


Figure 4. Blockade of Gal-9 ligand and inhibition of Gal-9-mediated suppressive mechanisms by HFB9-2. (A and B) HFB9-2 IC50 values for blocking Gal-9 binding to hTim3 or hCD44 determined by ELISA are 25nM and 40nM, respectively. (C) HFB9-2 prevents Gal-9-induced Th1 cell apoptosis with an IC50 value of 13nM determined by flow cytometry. (D) HFB9-2 suppresses the Gal-9-induced expansion of regulatory T cells with an IC50 value of 11nM determined by flow cytometry. Lactose as the positive control to abrogate lectin activity of Gal-9.

5. Developability Assessment of HFB9-2

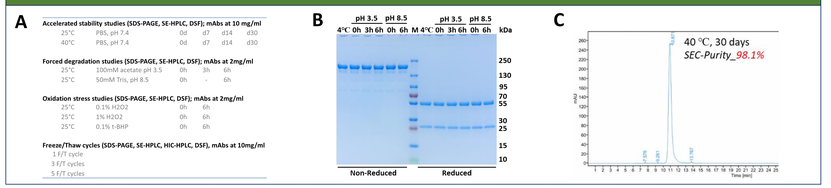


Figure 5. Developability assessment of HFB9-2 The stability of HFB9-2 was tested under different conditions including different temperatures, pH values, oxidation, and freeze/thaw cycles (A) by SDS-PAGE (B), and SE-HPLC (C). HFB9-2 demonstrated good stability under all tested conditions. Stability under different pH conditions shown in B and stability after 30 days at 40°C shown in C.

6. HFB9-2 Antitumor Activity in s.c. Syngeneic Tumor Model

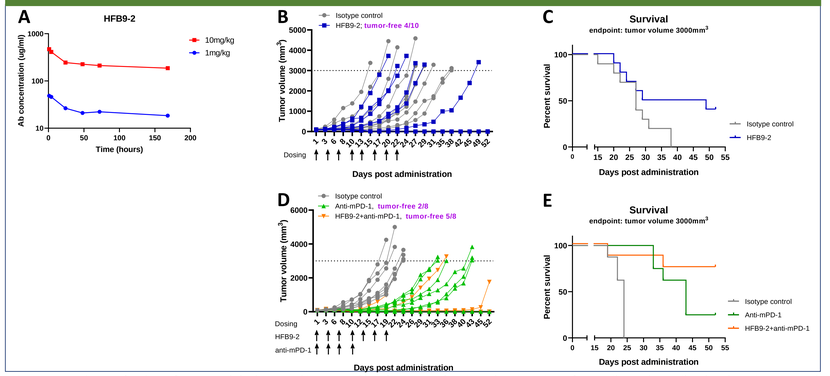


Figure 6. In vivo evaluation of HFB9-2. (A) Pharmacokinetic profile of HFB9-2 after i.v. administration of 10mg/kg and 1mg/kg. (B+C) Anti-tumor and survival effects of HFB9-2 (10mg/kg, Q3Dx8) in s.c. WEHI-164 syngeneic model. HFB9-2 treatment resulted in complete tumor rejection in 4 out of the 10 (40%) treated mice. (D+E) Anti-tumor and survival effects of HFB9-2 (10mg/kg, Q3Dx7) in combination with anti-mPD1 (RMP-14) antibody (10mg/kg, Q3Dx4) in s.c. WEHI-164 syngeneic model. Combination treatment resulted in complete tumor rejection in 5 out of 8 mice (62.5%) compared with 25% in the anti-mPD1 group.

CONCLUSION

Here we describe a humanized Gal-9 neutralizing antibody, HFB9-2, with the following properties:

- Sub-nanomolar affinity for human Gal-9
- Cross-reactivity to mouse and monkey Gal-9 orthologs
- Favorable developability and pharmacokinetic profiles
- Inhibitory activities of two key immunosuppressive functions of Gal-9, Th1 apoptosis and Treg expansion
- Anti-tumor efficacy in s.c. WEHI-164 syngeneic model

We also demonstrate, in collaboration with the Gustave Roussy institute, that Gal-9 levels are significantly higher in AML patients compared to healthy controls. Taken together we propose Gal-9 neutralizing antibody therapy as an approach to treat AML and other cancer types where Gal-9 expression associates with immunosuppression and resistance to immuno-therapies.