Abstract # 5626

Activation of Tumor Infiltrating Lymphocytes from Colorectal Cancer and Colorectal Liver Metastasis Patients by Anti-human PD-1 Antibody BGB-A317 in a 3D Spheroid System

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ABSTRACT

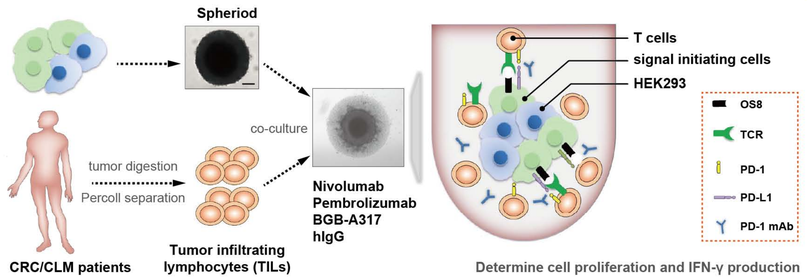
Background: Blockade of the PD-1 pathway with anti-PD-1 antibody, such as nivolumab (nivo) and pembrolizumab (pembro), has led to remarkable clinical responses in patients (Pts) with many different cancer types. BGB-A317, currently under clinical development, is a novel humanized IgG4 anti-PD-1 antibody. BGB-A317 has a unique binding signature to PD-1 with high affinity. Additionally, it is engineered to remove Fc gamma receptor (FcγR) binding, including FcγRI, FcγRIIA, FcγRIIB and FcγRIIIA. In this study, we investigated the activation of tumor infiltrating lymphocytes (TILs) from Pts with colorectal cancer (CRC) and colorectal liver metastasis (CLM) by BGB-A317 treatment and compared it with nivo and pembro.

Methods: TCR signal-initiating cells were used to generate a single spheroid. TILs, isolated from fresh CRC (23 Pts) and CLM (22 Pts) tumor tissues, were co-cultured with the spheroid in an *ex vivo* 3D assay to assess the immunomodulatory function of BGB-A317, nivo, and pembro. The treatment-mediated activation of TILs was evaluated by IFN-γ production and TILs proliferation. Flow cytometry and IHC were used to detect different immune cell subsets in tumor microenvironment (TME) of CRC and CLM Pts. The correlations between the immune cell subsets and the activation of TILs were also investigated.

Results: BGB-A317, nivo, and pembro all induced activation of TILs in a 3D *ex vivo* spheroid model. All three anti-PD-1 antibodies showed significant increases in IFN-γ and TILs proliferation in this model. Of note, in both CRC and CLM Pts, BGB-A317 treatment led to significantly higher IFN-γ production than that in nivo and pembro treated groups at concentration levels of 0.01, 0.1, 1 and 10 μg/mL. The TILs function was associated with a high density of $CD8^+$ T cells, but inversely correlated with the percentage of $CD11b^+$ myeloid cells and $CD68^+$ macrophages in CRC and CLM tumors. Of interest, BGB-A317 showed better activation of TILs in the liver metastasis TME where macrophages were more abundant.

Conclusions: These findings demonstrated that BGB-A317 exhibits potent TILs activation in *ex vivo* assay, which support its clinical development for the treatment of human cancers.

STUDY DESIGN



RESULTS

Activation of tumor infiltrating lymphocytes (TILs) in a 3D ex vivo spheriod model

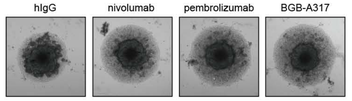


Figure 1. Spheroids and isolated TILs were co-cultured in ultra-low attachment (ULA) 96-well round-bottomed plates in the presense of 10 μg/mL hIgG or different PD-1 antibodies. Nivo, pembro and BGB-A317 treatment all significantly increased TIL proliferation.

Effect of three PD-1 mAbs on cytokine production from CRC and CLM patient TILs

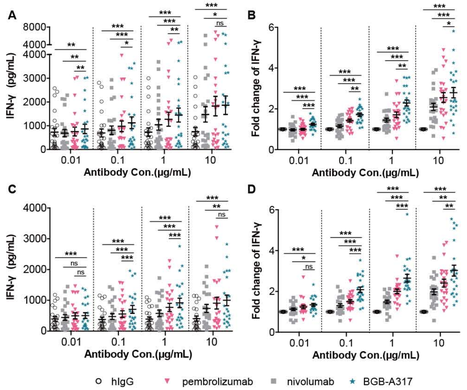


Figure 2. TILs obtained from 23 CRC patients (A, B) and 22 CLM patients (C, D) were co-cultured with speriods in the presense of 0.01, 0.1, 1 and 10 μg/mL hIgG or different PD-1 antibodies for 3-4 days.T cell function was evaluated by IFNγ release using ELISA. Absolute IFN-γ level (A, C) and fold change of IFN-γ (B, D) were plotted as mean±SEM. The differences between the mean values were tested for significance using paired t-test (ns: no significance; *: p<0.05; **: p<0.01; ***:p<0.001).

For TILs from both CRC and CLM patients, BGB-A317 induced significantly higher IFN-γ production than that nivo and pembro treatment at all tested concentrations, suggesting BGB-A317 could be more effective in blocking PD-1 mediated immune suppression.

Correlation between cytokine production of TILs and lymphocytes percentage in CRC and CLM patients

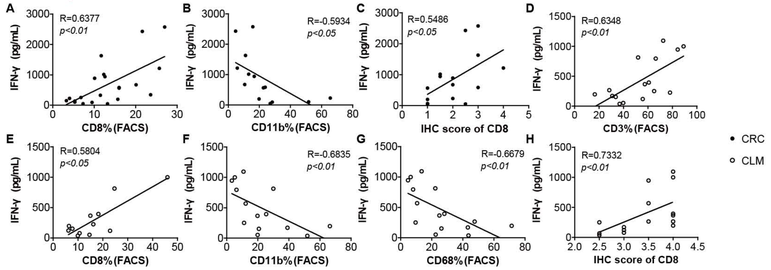


Figure 3. Expression of cell surface markers, such as CD3, CD8, CD11b, CD68 on TILs from CRC (filled circle) and CLM (open circle) was analyzed by FACS. CD8 expression in tumros was also determined by IHC staining and intensity was evaluated by IHC score, which ranges from 0 to 4.
The percentage of $CD3^+$ and $CD8^+$ T cells and IHC score of CD8 in tumors was postitively correlated with the IFN-γ production levels (1 μg/mL hIgG); and the percentage of $CD11b^+$ and $CD68^+$ cells was negatively correlated with IFN-γ production levels (1 μg/mL hIgG) from *ex vivo* 3D co-culture system.

BGB-A317 demonstrates better activation of TILs from CLM tumors where macrophages are more abundant

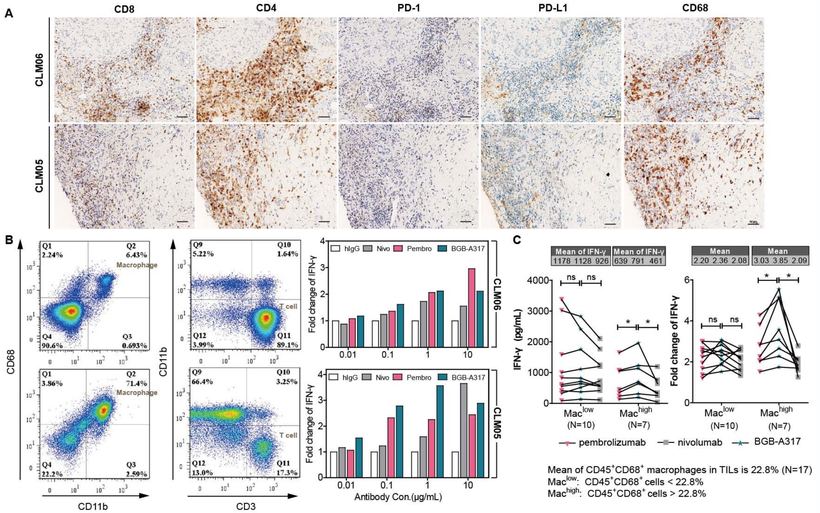


Figure 4. (A) Two representative CLM tumors showed either high infiltration of $CD4^+$ and $CD8^+$ T cells (CLM06) or $CD68^+$ macrophage (CLM05) by using IHC staining. (B) TILs isolated from the two tumors showed consistent profile of high percentage of $CD3^+CD11b^-$ T cells (CLM06) or high percentage of $CD68^+CD11b^+$ macrophages (CLM05). For TILs from CLM05 tumor, BGB-A317 induce much higher IFN-γ production than nivo and pembro treatment, especially at 0.1 and 1 μg/mL. (C) In CLM patients with higher amount of macrophages (above the average of 17 samples), higher IFN-γ production was observed in BGB-A317 vs. nivo & pembro treatment (10 μg/mL). The differences between the mean were tested for significance using paired t-test (ns: no significance; *: p<0.05)

SUMMARY

- BGB-A317, nivo, and pembro all induced activation of TILs in a 3D *ex vivo* TIL/spheroid co-coulture model, indicated by enhanced lymphocyte proliferation and IFN-γ production.
- *Ex vivo* function of TILs was associated with a high density of $CD8^+$ T cells, but inversely correlated with the percentage of $CD11b^+$ myeloid cells and $CD68^+$ macrophages in CRC and CLM tumors.
- BGB-A317 exibited high affinity to the target receptor PD-1, indicated by better activity in enhancing cytokine production in TILs from both CRC and CLM tumors than nivo and pembro.
- BGB-A317 is engineered to remove FcγR binding, which minimizes the antibody-dependent cellular phagocytosis (ADCP) activity, allowing it to be more effective in tumor microenvironment where macrophages are abundant.