Background

Cancer immunotherapies, including immune checkpoint inhibitors, CAR-T, cancer vaccines and bispecific antibodies, have been brought to spotlight in recent years as several therapeutic strategies targeting the immune system have produced exciting clinical results. Bispecific antibody typically play dual roles in blocking the immune checkpoint and redirecting/re-boosting the function of the immune effector cells. Blinatumomab belongs to CD3 bispecific T cell engager (CD3 BiTE), which was engineered to harbor two arms binding with CD3 and CD19 simultaneously and direct CD8+ T cells to specifically recognize CD19 positive lymphoma cells to execute cytotoxicity. Approval of Blinatumomab for patients with relapsed/refractory B cell acute lymphoblastic leukemia (ALL) has driven remarkable increase in combination studies of Blinatumomab with other immunotherapies such as checkpoint inhibitors.

In this study, we developed CD8+ T cytotoxic system targeting different B lymphoma cell line and fully validated the function of Blinatumomab in promoting target tumor cell lysis by primary CD8+ T cells. In addition, we established a mixed lymphocyte and tumor system to mimic physiological TME to dissect the combinational role of Nivolumab and Blinatumomab.

Results

CD8+ T cell co-culture with Raji cell in the presence of Blinacito. Blinacito can promote T cell activation by increase CD25 expression and promote T cell proliferation. In addition, Blinacito promote Raji cell lysis in a dose dependent manner.

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Methods

CD8+ T Cytotoxicity Assay

CD8+ T cell isolated from PBMC using negative selection kit. Target cancer cell (Raji, Daudi) was labeled with CellTrace™ Violet and then co-culture with CD8+ T for 3 days. CD8+ T cell was analyzed of proliferation, CD25 and PD1 expression by FACs.

Cancer cell was analyzed of lysis ratio

Supernatant was analyzed for INFγ

PBMC Cancer MLR:

PBMC was labeled with CellTrace™ Violet

PBMC and Raji was co-cultured in the Ratio of 10:1 (PBMC: Raji) for 48 hours.

T cell was analyzed by flow and supernatant was analyzed for INFγ by ELISA

Figure 1 Blinacito mediate T cell activation and target cell lysis in CD8+ T and Raji co-culture system

Figure 2 Blinacito mediate T cell activation and target cell lysis in CD8+ T and Daudi co-culture system

Figure 3 Opdivo further promote CD8+ T cell proliferation and activation in the context of Blinacito treatment

CD8+ T cell co-culture with Daudi cell in the presence of Blinacito. Blinacito can promote T cell activation by increase CD25 expression and promote T cell proliferation. In addition, Blinacito promote Daudi cell lysis in a dose dependent manner.

2M Raji mixed with 3M hPBMC, co-inoculated into right flank of 6-8ws female NOG mice. Dosing was started when average tumor size reached to approx. 50mm3.

Blinacito significantly inhibit tumor growth compared to Opdivo only group. Tumor from Blinacito and Opdivo combo group share the similar growth curve with Blinacito only group. But at early time window, tumor from combo group display slower growth dynamic.

Figure 4 Effect of Blinacito and combination with opdivo in Raji humanized mouse model.

Summary

• Blinacito significantly promote T cell activation and increase cancer cell lysis in the in-vitro human PBMC model

• The effect of Opdivo under treatment of Blinacito can depend on the concentration of Blinacito and also related to PBMC/Raji ratio, suggesting combinatorial treatment of opdivo with Blinacito should take the consideration of Blinacito dosage and also the tumor micro-environments of patients. With more Macrophage/DC infiltration, more chance of get synergistic effect.

Reference