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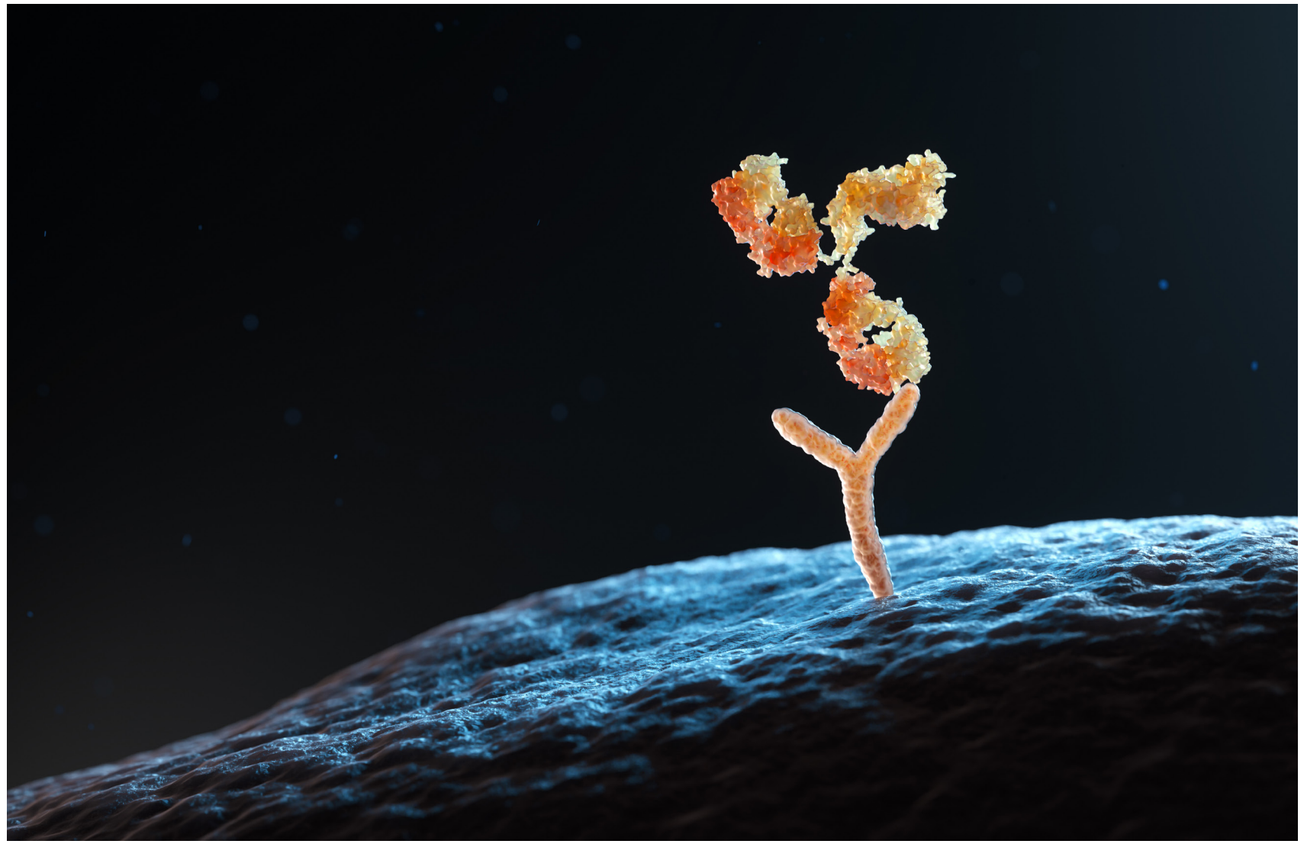

Pharmacodynamic Effects and Toxicity Study of Multispecific Antibodies in a Humanized Mouse Model Bearing Tumors

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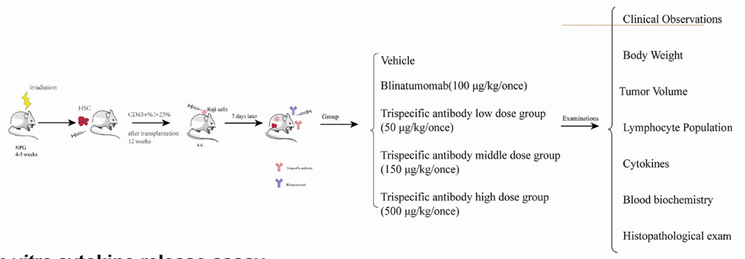
INTRODUCTION

T-cell engagers (bispecific antibodies) can bind both tumor cells and immune cells such as T-cells, and direct immune cells to kill tumor cells by the dual binding. However, the bispecific antibodies are easily depleted, which cause activated T-cells to become unresponsive shortly after exposure due to lack of costimulatory molecules. Based the above observation, tri-specific antibody with the addition of a costimulatory molecule was created to avoid incapacity of T-cells activated by a single signal. This is expected to improve T-cell killing efficacy to tumor cells. This study evaluated the anti-cancer efficacy and potential toxicity of CD3/CD20/CD28 antibodies and Blinatumomab analog monoclonal antibodies on a Raji tumor-bearing NPG mouse model with human hematopoietic stem cells (HSC) reconstitution, as well as their *in vitro* effect on human peripheral blood mononuclear cells (PBMCs) activation.

MATERIALS & METHODS

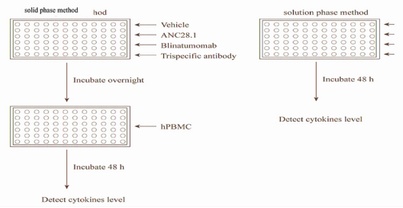
In vivo study design

NPG mice (Beijing Vitalstar Biotechnology Co., Ltd.) were transplanted with human hematopoietic stem cells(HSCs) by intravenous injection at 4-5 weeks of age after irradiation, and the proportion of CD45+ cells in the peripheral blood of mice was measured 12 weeks after transplantation. Animals with the CD45+ cell proportion greater than 25% were used in this experiment. The operation of animals in this study was approved by the Institutional Animal Care and Use Committee (IACUC) at JOINN Laboratories(Suzhou) Co., Ltd.



In vitro cytokine release assay

Solid phase: Vehicle, Blinatumomab and test articles were plated overnight, after which PBMCs were added and incubated for 48 hrs. The supernatant was collected for analysis.
Solution phase: After co-incubation of vehicle, Blinatumomab or test articles and PBMC for 48 h, the supernatant was collected for analysis.



RESULTS

Clinical observations and body weight

By day 28 after dosing, 3/10 and 1/10 animals in the trispecific antibody test article middle and high dose groups died, with pale limbs and ears, emaciation and other symptoms observed before death.

Combined with lymphocyte population data, it was speculated that the animal death may be related to graft-versus-host disease (GVHD) caused by the high initial amounts of lymphocytes in the animals (Figure 1).

Anti-tumor activity

Due to the proliferation of human lymphocytes in HSC-NPG mice, the tumor volume of animals in vehicle group decreased since day 10. On several time points, the tumor growth was significantly inhibited by Blinatumomab and all doses of tri-specific antibodies (Figure 2).

Lymphocyte Population

During the study, the proportion and absolute number of CD45+ CD20+ B cells were decreased after administration of the Blinatumomab and tri-specific antibodies (Figure 3).

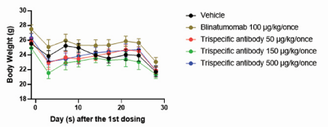


Fig 1. Body weight of each group during the experimental period. N=7~10

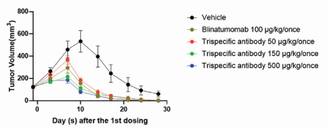


Fig 2. Tumor Volume of each group during the experimental period. N=7~10

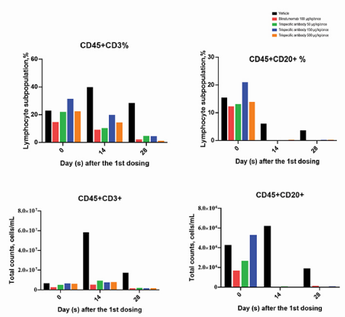


Fig 3. The Lymphocytes population of each group during the experimental period. N=7~10

Cytokines release

In vivo cytokine release

During the experiment, the cytokine levels in each dose group of tri-specific antibody were higher than those in the Blinatumomab group (Fig 4).

In vitro cytokine release

The results of *in vitro* cytokine release assay (solid phase) indicated that the cytokine levels in each dose of tri-specific antibody were higher than those of Blinatumomab (Fig 5). The result of solution phase were the similar to those of the solid phase (data not shown).

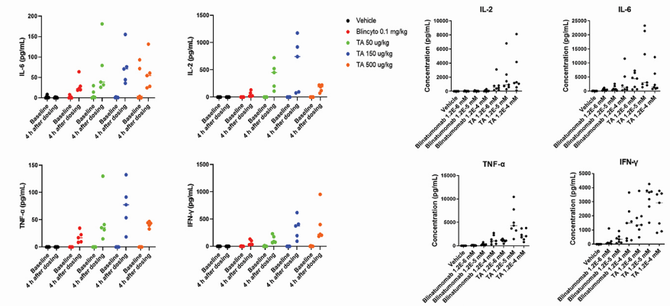


Fig 4. The level of specific cytokines increased in the tri-specific antibody groups compared to control or Blinatumomab groups 4 hrs after dosing. N=5

Fig 5. The level of specific cytokines measured in vitro solid phase increased in the tri-specific antibody groups compared to control or Blinatumomab. N=6

Organ weight

On day 28, compared with vehicle group, animals in Blinatumomab group and tri-specific antibody group did not show abnormality of organs except spleen. Compared with vehicle group, organ weight and organ-to-brain weight ratio were decreased in Blinatumomab group, low and medium dose groups of tri-specific antibody, and increased in high dose group of tri-specific antibody.

Organ weight

Group		Brain	Spleen	Liver	Kidney	Adrenal Gland
Vehicle	Mean±SD	0.466±0.024	0.077±0.035	0.930±0.124	0.277±0.026	0.087±0.002
	n	10	10	10	10	10
Blinatumomab group	Mean±SD	0.474±0.029	0.044±0.023	0.982±0.073	0.297±0.028	0.089±0.005
	n	10	10	10	10	10
Trispecific antibody low dose group	Mean±SD	0.470±0.013	0.063±0.042	0.967±0.066	0.281±0.025	0.087±0.002
	n	10	10	10	10	10
Trispecific antibody middle dose group	Mean±SD	0.467±0.016	0.060±0.031	0.931±0.081	0.273±0.021	0.087±0.004
	n	7	7	7	7	7
Trispecific antibody high dose group	Mean±SD	0.467±0.008	0.480±0.095	1.067±0.177	0.292±0.026	0.087±0.002
	n	9	9	9	9	9

Organ weight/brain weight ratio

Group		Spleen	Liver	Kidney	Adrenal Gland
Vehicle	Mean±SD	0.154±0.101	2.036±0.201	0.595±0.057	0.016±0.005
	n	10	10	10	10
Blinatumomab group	Mean±SD	0.093±0.057	2.070±0.173	0.625±0.054	0.020±0.014
	n	10	10	10	10
Trispecific antibody low dose group	Mean±SD	0.143±0.140	2.060±0.152	0.599±0.053	0.015±0.004
	n	10	10	10	10
Trispecific antibody middle dose group	Mean±SD	0.129±0.064	1.992±0.150	0.585±0.048	0.016±0.003
	n	7	7	7	7
Trispecific antibody high dose group	Mean±SD	0.172±0.205	2.282±0.367	0.625±0.052	0.014±0.005
	n	9	9	9	9

Histopathological examination

Under the conditions of this assay, four animals in the tri-specific antibody middle and high groups were found dead but the cause of death was unclear and gross observation did not reveal significant changes. Both dead animals and animals euthanized on day 28 were found with pathological changes related to Blinatumomab or tri-specific antibody in spleen by microscopic observation, mainly manifested as decreased lymphocytes in white pulp (Fig 6).

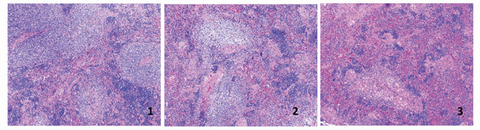


Fig 6. 1-Vehicle group, no significant abnormal change observed in spleen; 2- Blinatumomab group, lymphocytes decreased in splenic white pulp; 3- tri-specific antibody high dose group, lymphocytes decreased in splenic white pulp, H&E staining, 100X magnification.

CONCLUSION

The results showed that both tri-specific antibody and Blinatumomab could induce T cell activation *in vitro*, significantly inhibit the proliferation of tumor cells *in vivo*, induce cytokine release and T cell depletion, and target-related histopathological changes were observed in the spleen. Since CD28 costimulatory factor is added to the tri-specific antibody molecule, its killing effect on tumor cells may be higher than that of Blinatumomab, but the risk of causing cytokine release may also be increased.