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TITLE:

A Human Peripheral Blood Mononuclear Cell (PBMC) Engrafted Humanized Xenograft Model for Translational Immuno-oncology (I-O) Research

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KEYWORDS:

immuno-oncology (I-O), peripheral blood mononuclear cell (PBMC), Tislelizumab, BGB-A317, immunodeficient, humanized, cyclophosphamide (CP), patient-derived xenograft (PDX)

SHORT ABSTRACT:

We describe a human peripheral blood mononuclear cell (PBMC) - based humanized xenograft mouse model for translational immuno-oncology research. This protocol could serve as a general guideline for establishing and characterizing similar models for I-O therapy assessment.

LONG ABSTRACT:

The discovery and development of immuno-oncology (I-O) therapy in recent years represents a milestone in the treatment of cancer. However, treatment challenges persist. Robust and disease-relevant animal models are vital resources for continued preclinical research and development in order to address a range of additional immune checkpoints. Here, we describe a human peripheral blood mononuclear cell (PBMC) - based humanized xenograft model. BGB-A317 (Tislelizumab), an investigational humanized anti-PD-1 antibody in late-stage clinical development, is used as an example to discuss platform set-up, model characterization and drug efficacy evaluations. These humanized mice support the growth of most human tumors tested, thus allowing the assessment of I-O therapies in the context of both human immunity and human cancers. Once established, our model is comparatively time- and cost-effective, and usually yield highly reproducible results. We suggest that the protocol outlined in this article could serve as a general guideline for establishing mouse models reconstituted with human PBMC and tumors for I-O research.

INTRODUCTION:

Immuno-oncology (I-O) is a rapidly expanding field of cancer treatment. Researchers have recently started to appreciate the therapeutic potential of modulating functions of the immune system to attack tumors. Immune checkpoint blockades have demonstrated encouraging activities in a variety of cancer types, including melanoma, renal cell carcinoma, head and neck,

lung, bladder and prostate cancers[1,2]. Contrary to targeted therapies that directly kill cancer cells, I-O therapies potentiate the body's immune system to attack tumors[3].

To date, numerous relevant I-O animal models have been established. These include: 1) mouse tumor cell lines or tumor homograft in syngeneic mice; 2) spontaneous tumors derived from genetically engineered mouse (GEM) or carcinogen-induction; 3) chimeric GEMs with the knock-in of human drug target(s) in a functional murine immune system; and 4) mice with reconstituted human immunity transplanted with human cancer cells or patient-derived xenografts (PDXs). Each of these models have obvious advantages as well as limitations, which have been described and reviewed extensively elsewhere[4].

Reconstitution of human immunity in immunodeficient mice have been growingly appreciated as a clinically relevant approach for translational I-O research. This is usually achieved through either 1) engraftment of adult immune cells (e.g., peripheral blood mononuclear cells (PMBC))[5,6], or 2) engraftment of hematopoietic stem cells (HSC) from, for example, umbilical cord blood or fetal liver[7,8]. These humanized mice could support the growth of human tumors, thus allowing the assessment of I-O therapies in the context of both human immunity and human cancers. Despite the advantages, applications of humanized mice in I-O research were usually hindered by several concerns, such as long model development time and considerably high cost.

Here, we describe a human PBMC-based model that could be widely applied for translational I-O studies. This model is comparatively time- and cost-effective with high reproducibility in efficacy studies. It has been used in-house for the evaluations of several I-O therapeutics currently under preclinical and clinical development. BGB-A317 (Tislelizumab), an investigational humanized anti-PD-1 antibody[9] , is used as the example to discuss model development, characterization, and possible applications for anti-tumor efficacy analyses.

PROTOCOL:

All procedures performed in studies involving human participants were in accordance with the ethical standards of BeiGene and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving animals were approved by the Internal Review Board at BeiGene. This protocol has been specifically adjusted for the evaluation of BGB-A317 (Tislelizumab) in humanized NOD/SCID mice.

1. Establishment of human PBMC-based model

1.1. Myeloablation of NOD/SCID mice using cyclophosphamide: determination of optimal doses

1.1.1. Purchase female NOD/SCID mice (6-8 weeks).

NOTE: All mice involved in this study were female.

1.1.2. Prepare cyclophosphamide (CP) at different doses (50, 100 and 150 mg/kg) in saline. Prepare disulfiram (DS) in 0.8% Tween-80 in saline at 125 mg/kg.

NOTE: Different concentrations of CP were prepared to enable administration of equal volumes of drug solution to mice getting different doses of CP.

1.1.3. Treat the animals with CP (i.p.) and DS (p.o.) once a day for 2 days. Give DS (p.o.) 2 h after each dose of CP.

NOTE: DS decreases the urotoxicity of CP in mice, and CP combined with DS has been suggested to have longer-lasting neutropenia than animals treated with CP alone[10]. The dose regimen of CP might need to be pre-determined prior to actual studies and was found to vary slightly between different immunodeficient mouse strains.

1.1.4. Collect blood samples from the orbital venous sinus and transfer to EDTA-K coated tubes on ice on day 0 (1 h before the 1st dose), day 2 (24 h after the 2nd dose) and day 4 (72 h after the 2nd dose).

1.1.5. Examine the myeloablation effect after CP and DS treatment by FACS. Use rat anti-mouse CD11b (M1/70), rat anti-mouse Ly6C (HK1.4,) and rat anti-mouse Ly6G (1A8) for gating CD11b^{+} Ly6G^{high} as neutrophils, CD11b^{+}Ly6C^{high} as monocytes[11,12].

1.1.6. Record body weight and health conditions of the mice daily for one week. The optimal dose of CP and DS is determined as the regimen that results in maximum depletion of neutrophils and monocytes without causing severe toxicity to mice.

1.2. Human PBMC transplantation and tumor engraftment: model set-up

1.2.1. Isolate human PBMCs from healthy donors by density gradient centrifugation according to the manufacturer's instructions.

1.2.2. Pre-treat the mice with CP and DS as indicated by step 1.1.2 and 1.1.3 to increase transplantation efficiency.

1.2.3. 20 to 24 h after the second dose of CP and DS, inject human tumor cell line such as A431 cells (ATCC, 2.5 × 10^6) and 5 × 10^6 isolated PBMCs (mixed in a total of 200 µL phosphate-buffered saline (PBS) containing 50% Matrigel), or tumor fragments (3×3×3 mm^3, in a total volume of 200 µL PBS containing 50% Matrigel) and 200 µL of 5 × 10^6 PBMCs (100 µL each to the left and right side of engrafted tumor fragment) (s.c.) subcutaneously in the right flank of the animals.

1.2.4. Measure primary tumor volume and record twice a week for 4-6 weeks.

NOTE: The mice will be euthanized once their body weights lose over 20% or their tumor volume

reaches 2000 mm^3 or the tumor is ulcerated.

1.2.5. Euthanize the mice in gas chambers with carbon dioxide. Collect the whole tumor tissues in sacrificed mice with ophthalmic scissors and process them for histology and immunohistochemistry (IHC) analysis. Examine the Human CD8, PD-1 and PD-L1 expressions in these tissues. See protocol step 4.

2. PBMC donor screen

2.1. Screen a panel of PBMC donors due to the anticipated variations resulted from PBMCs collected from individuals. Use A431 cells co-injecting with PBMCs from different donors according to the procedures as indicated by step 1.2.

NOTE: Over 50 healthy PBMC donors were screened in the study in order to obtain enough number of suitable donors. Researchers who would like to adopt this protocol might decide on their own how many healthy PBMC donors to be screened, based on the design of the planned studies.

2.2. Monitor tumor volume twice a week by measuring with a caliper.

NOTE: Tumor growth rate may vary with PBMC from different donors.

2.3. Collect the tumor tissues at an average volume of 200-500 mm^3 and process them for histology and immunohistochemistry (IHC) analysis. Examine human CD8, PD-1 and PD-L1 expressions. See step 4 for detailed protocol.

2.4. Select PBMC donors that result in moderate tumor growth (tumor volume > 200 mm^3 14 days post inoculation) and relatively high PD-1, PD-L1 and CD8 expressions (mean IHC scores > 2). See step 4 for detailed IHC scoring protocol.

3. Human cancer cell line and PDX screen

3.1. Screen cell lines and PDXs according to the procedures stated in step 1.2, to evaluate tumor growth rate, human PD-L1 expression of the tumors and immune cell infiltrations.

NOTE: Over 30 human cancer cell lines and over 20 PDXs of different cancer types were screened by the authors. Data of selected tumor models were shown in the results section.

4. Immunohistochemistry (IHC)

4.1. Harvest as indicated by step 1.2.5 and fix tumor tissues by immersing in formalin. Dehydrate and embed fixed tissues in paraffin. Section the fixed tissues at 3 µm and place them on polylysine-coated slides.

4.2. Deparaffinize in xylenes three times 7 min each. Hydrate the sections through graded alcohols: 100% ethanol twice for 3 min each, followed by 90%, 80% and 70% ethanol in turn for 3 min each. Rinse by deionized H_2O three times and remove excess liquid from the slides.

4.3. Perform antigen retrieval by placing the slides in a container and cover with 10 mM sodium citrate buffer (pH 6.0), or Tris-EDTA (pH 9.0). Heat the slides container by microwave for 3 min. Boil in a water bath at 95 °C for 30 min and then cool down to room temperature. Rinse by deionized H_2O three times and aspirate excess liquid from the slides.

4.4. Block the sections by 3% bovine serum albumin in PBS for 1 h and 0.3% H_2O_2 solution in PBS for 10 min. Stain by antibodies against human CD8 (EP334), PD-1 (NAT105,) and PD-L1 (E1L3N) at 4 °C overnight, and HRP conjugated 2nd antibodies at RT for 1 h. Drop the substrate DAB (3,3'-diaminobenzidine) onto the slides and control the reaction time (seconds to minutes) by monitoring the brown color from microscope.

4.5. Cover the slides with neutral balsam after immersing the slides in 0.5% hydrochloric acid alcohol and 0.5% ammonia water in turn for 5 s each, then in 80%, 90% and 100% ethanol in sequence for 3 min each, and finally in xylenes using three changes for 5 min each. Detect the antibodies by observing the brown color of DAB using microscope.

NOTE: Human CD8 and PD-1 expression on tumor-infiltrating leucocytes (TIL) were assessed by assigning an expression score on a 5-point scale (IHC score, range 0–4) at high objective magnification (20X, 40X). 0, absent; 1, weak intensity/ less than 20% cells; 2, weak-to-moderate intensity/ 20%–50% cells; 3, moderate-to-strong intensity/ 50%–80% cells; 4, strong intensity/ more than 80% cells. Human PD-L1 staining within tumor cells was scored using an adjusted scoring system on a 5-point scale (IHC score, range 0–4) because of its relatively diffused signal. 0, absent; 1, weak intensity/ less than 10% cells; 2, weak-to-moderate intensity/10%–30% cells; 3, moderate/ 30%–50% cells; 4, strong intensity/ more than 50% cells.

5. In vivo efficacy and pharmacodynamics studies in humanized PBMC-NOD/SCID xenograft models

5.1. Pre-treat NOD/SCID mice as indicated by step 1.1.3. In brief, treat the mice with 100 mg/kg CP (i.p.) and 125 mg/kg DS (p.o.) once a day for 2 days.

5.2. 20 to 24 h after the second dose, inject subcutaneously (s.c.) with indicated number of human cancer cells and 2.5-5 × 10^6 PBMCs (a total of 200 µL cell mixture in 50% Matrigel) in the right front flank of animals.

NOTE: The number of PBMC used for any individual mouse in one single study should be the same. However, due to variations in the availability of total isolated PBMC at the time of each study, the authors have chosen to use 2.5 x 10^6, 4 x 10^6, or 5x10^6 PBMC at different studies. Although this 2-fold difference in the administered amount of PBMCs might affect the degree of humanization, the authors do not observe significant differences in evaluating anti-tumor

efficacies of the tested immunotherapies.

5.3. For PDXs engraftment, inject subcutaneously tumor fragments (3×3×3 mm^3) in the right front flank of animals. Inject subcutaneously 200 µL of 5 × 10^6 PBMCs (100 µL each side) to the left and right of engrafted tumor fragment.

NOTE: PDX tumor tissues were administered in a Matrigel solution, same as described for the cell line models.

5.4. On the day of cell inoculation, randomly group the animals and treat as the planned study protocol. Assess the anti-tumor activity of candidate drugs, BGB-A317 (QW, i.p.) in this case, at the indicated doses in various tumor models.

NOTE: The three human cancer cell lines (i.e., A431 (epidemoid carcinoma), SKOV3 (ovarian cancer) and SK-MES-1 (lung cancer)), as well as two PDX models (i.e., BCLU-054 (lung cancer) and BCCO-028 (colon cancer)), are considered good tumor models for I-O therapy evaluation in this humanized mouse model.

5.5. Measure primary tumor volume twice every week, using a caliper.

NOTE: Graft-vs-host disease (GvHD)-associated clinical observations and body weights loss were observed around 4-6 weeks post PBMC engraftment in our studies, allowing a 1-2 months window for therapeutic efficacy evaluations.

5.6. For the pharmacodynamics analysis of tumor infiltrated immune cells, cut the tumor tissues into small pieces and digest them with collagenase type I (1 mg/mL) and DNase I (100 µg/mL) in RPMI1640 plus 5% fetal bovine serum (FBS) for 30 min at 37 °C. Pass the digested tissues through 40 µm cell strainers to obtain single cell suspensions.

5.7. Wash the cells and adjust cell number to a concentration of 1x10^7 cells/mL in ice cold FACS Buffer (PBS, 1% FBS) in 96-well round bottom plates. Wash the cells by centrifuging and block them by adding 20 µg/mL human IgG for 30 min, followed by staining with anti-human CD3 (HIT3a), CD8 (OKT8) and PD-1 (MIH4) antibodies at 4 °C for 30 min. Then subject the stained samples to flow cytometry and analyze using guavaSoft 3.1.1.

REPRESENTATIVE RESULTS:

Following the procedures presented here, a PBMC-based humanized xenograft model was successfully established. In brief, CP myeloablation effects in NOD/SCID mice was determined by flow cytometry analysis of neutrophil and monocyte populations post CP and DS treatment (**Figure 1**). 100 mg/kg CP plus 125 mg/kg DS was determined as the optimal dose and used in later studies as the regimen results in maximum depletion of neutrophils and monocytes without causing severe toxicity to mice. Next, human PBMC and tumor transplantation was performed. Presence of human immune cell infiltrates in the tumor microenvironment was verified by IHC (**Figure 2**).

A panel of PBMC donors were screened in vivo to ensure relatively high immune cell infiltrations in the tumor microenvironment and acceptable tumor growth rate (section 2, **Figure 3A**). Meanwhile, over 30 human cancer cell lines as well as over 20 PDXs of different cancer types were screened to evaluate tumor growth rate, tumor PD-L1 expression and immune cell infiltrations (section 3). Representative results were shown in **Figure 3B**.

These PBMC-engrafted humanized mice were then used to examine the anti-tumor activity of BGB-A317. Human PBMCs from selected healthy donors were co-injected with human tumor cells (A431, SKOV3 and SK-MES-1) or primary tumor tissue fragments derived from cancer patients (BCCO-028 and BCLU-054) subcutaneously. The mice were treated, as indicated in **Figure 4**, with BGB-A317 or PBS intraperitoneally once a week from the day of tumor implantation. In all abovementioned models, BGB-A317 demonstrated significant anti-tumor activities (**Figure 4**).

FIGURE AND TABLE LEGENDS:

Figure 1. Myeloablation of NOD/SCID mice using cyclophosphamide (CP) and disulfiram (DS). (**A**) Gating strategy used to identify myeloid cell subsets including neutrophils and monocytes. (**B**) Representative results of myeloid cell (CD11b+), neutrophil (CD11b+Ly6G+) and monocyte (CD11b+Ly6C+) numbers upon different dosages of CP treatment. The boxes represent the 75th, 50th and 25th percentile of the values. The top and bottom lines represent maximum and minimal data points within the 1.5x IQ (inter quarter) range, respectively. n = 3 for the vehicle group and n = 6 for CP and/or DS treated groups.

Figure 2. Human PBMC transplantation and tumor engraftment. (**A**) Schematic diagram showing the general workflow of PBMC-based humanized xenograft model. (**B**) Tumor growth of A431 cells upon subcutaneous co-injection with donor PBMC with the indicated conditions (data represents mean tumor volume ± SEM, n = 6). (**C**) IHC analysis of tumors developed in mice treated with or without CP+DS.

Figure 3. PBMC donor and human cancer cell line screen. (**A**) Representative summary data from PBMC donor screen. PBMC were mixed with A431 cells and inoculated subcutaneously in humanized NOD/SCID mice (see step 2). Each dot represents the mean data value of 3 mice engrafted with PBMCs from 1 donor. (**B**) Representative results from human cancer cell line screen. PBMC from selected donors were co-injected with A431, SK-MES-1 or SKOV3 cells. Data represents mean tumor volume ± SEM collected from 3 mice, 14-day post inoculation of the indicated cell lines. Mean IHC score ± SEM represents the average expression of human CD8, PD-1, and PD-L1 of all 3 mice.

Figure 4. Anti-tumor activities and pharmacodynamics analysis of BGB-A317 in PBMC-based humanized xenograft model. The anti-tumor activity of BGB-A317 at indicated doses (i.p., QW) was assessed using human cancer cell lines (**A**) A431 (with $5x10^6$ PBMC), (**C**) SKOV3 (with $5x10^6$ PBMC), (**D**) SK-MES-1 (with $5x10^6$ PBMC), and patient-derived xenografts (PDXs) (**E**) BCLU-054 (with $5x10^6$ PBMC) and (**F**) BCCO-028 (with $5x10^6$ PBMC). PBMC from selected healthy donors

and corresponding tumor cells were co-injected subcutaneously into humanized NOD/SCID mice (n = 8 to 10). (**B**) Quantification of tumor-infiltrating hCD8+ and hCD8+hPD-1+ cells in BGB-A317 treated A431 tumors. n=8-10 animals per group in A, and C to F, n=4-6 animals per group in B; data represents mean ± SEM. The significance was evaluated using a two-tailed unpaired Student's t-test under the assumption of unequal variance.

DISCUSSION:

Our knowledge of cancer development and progression has advanced significantly in recent years, with focus on a comprehensive understanding of both the tumor cells and its associated stroma. Harnessing the host immune mechanisms could induce a greater impact against cancer cells, representing a promising treatment strategy. Murine models with intact mouse immune systems, such as syngeneic and GEM models, have been widely used to study checkpoint-mediated immunity. Efficacy assessments using these models depend largely on surrogate anti-mouse target antibodies[13,14]. However, inherent differences between human and murine immune systems and the lack of some human targets in murine models limit preclinical studies of I-O anti-tumor effects[15,16]. Therefore, robust mouse models that include both human immune cells and human tumors are urgently desired, which will significantly improve the translation and development of novel I-O therapeutics.

Here, we describe a human PBMC-based xenograft mouse model that could potentially be widely used for translational I-O studies. PBMC donor as well as cancer cell line/PDX screens are critical to ensure robustness and reproducibility of in vivo efficacy studies. PBMC donors were screened in vivo to ensure successful human tumor and immune cell engraftment. Meanwhile, over 50 cell lines and PDXs of various human cancer origins were screened to evaluate tumor growth rate, human PD-L1 expression, and immune cell infiltrations. Our analyses suggest that about 20% of cancer cell lines and PDXs examined demonstrate acceptable tumor growth rate while at the same time having relatively high TILs and PD-L1 staining, which are considered good models for I-O efficacy evaluations.

HLA matching is routinely used in the clinic to match patients and donors for organ or marrow transplants[17]. The authors, however, have only performed limited characterization on HLA typing, and this remains an interesting topic to be investigated in future studies. The authors would like to note that a PBMC donor might be suitable for one cancer cell line/ PDX but not ideal for others. Therefore, PBMC donors might need to be screened for each cancer model to ensure optimal results.

Engraftment of human PBMC into NOD/SCID or NSG mice invariably leads to a xenogeneic graft-vs-host disease (xGvHD), a post-transplant disorder that results from immune-mediated attack of recipient tissue by donor T cells[18,19]. Clinical observations commonly associated with xGVHD have been observed in our humanized model, such as erythema, hunched posture, weight loss and mortality (data not shown). These phenotypes were usually observed towards the end of our studies, usually at 1-2 months post engraftment, indicating the propagation and infiltration of human T cells in xGVHD target organs. This allows a 1-2 months window for therapeutic I-O

therapy evaluations. Several approaches have been utilized to decrease mouse innate immunity and enhance human immune cell engraftment[20,21]. For example, NSG mice defective in murine MHC Class I and Class II expression support engraftment of functional human T cells in the absence of acute xGvHD following injection of PBMC[22].

NOD/SCID mice were used in this protocol. Mice homozygous for the SCID mutation have impaired T and B cell lymphocyte development and the NOD background additionally results in deficient natural killer (NK) cell function[20]. Other more highly immunodeficient mice, such as the NSG (The Jackson Laboratory), NCG (Charles River) and NOG (CIEA) strains, have been established. When engrafted with PBMCs, these mice have been shown develop human immune cells and form an environment that resembles the human immune system[23,24]. Alternatively, these mice could be engrafted with CD34+ human hematopoietic progenitor cells (HPCs) and display more sustained T cell differentiation and maturation[25]. In addition, next generation immunodeficient mouse models with further genetic modifications have been established to support better human myeloid lineage development and increased engraftment efficiency (refer to the variants portfolio webpage of The Jackson Laboratory and Taconic Biosciences).

More details of using these new strains for human immunity reconstitution are pending further investigations. Nevertheless, the protocol outlined in this article could serve as a general guideline of establishing and characterizing immunodeficient mouse models reconstituted with human PBMC. Three human cancer cell lines and two human patient-derived xenografts, covering a range of cancer types, are demonstrated in this article, suggesting the potential broad applications of our protocol in translational I-O studies. Most humanized PBMC models, to our knowledge, have chosen IV or IP as the route of injection[26,27]. Our models instead provide partially reconstituted human immunity in human tumor bearing mice through subcutaneously admixing of human PBMC with cancer xenografts. This approach provides a rapid and cost-effective, yet highly reproducible alternative to full stem cell reconstitution (e.g., CD34+ hematopoietic stem cell-engrafted humanized mice). Our model has been proved to be useful for evaluating T cell-engaging cancer immunotherapies, particularly when working on short timelines or to select agents before moving to a more complex multi-lineage immunity model.

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DISCLOSURES:

All authors have ownership interest in BeiGene. Tong Zhang and Kang Li are inventors on a patent covering BGB-A317 described in this study.

REFERENCES:

1 Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer.* **12** (4), 252-264, (2012).

2 Postow, M. A., Callahan, M. K. & Wolchok, J. D. Immune Checkpoint Blockade in Cancer Therapy. *Journal of Clinical Oncology.* **33** (17), 1974-1982, (2015).

3 Li, Z. & Kang, Y. Emerging therapeutic targets in metastatic progression: A focus on breast cancer. *Pharmacology & Therapeutics.* **161** 79-96, (2016).

4 Li, Q. X., Feuer, G., Ouyang, X. & An, X. Experimental animal modeling for immuno-oncology. *Pharmacology & Therapeutics.* **173** 34-46, (2017).

5 Fisher, T. S. *et al.* Targeting of 4-1BB by monoclonal antibody PF-05082566 enhances T-cell function and promotes anti-tumor activity. *Cancer Immunology, Immunotherapy.* **61** (10), 1721-1733, (2012).

6 McCormack, E. *et al.* Bi-specific TCR-anti CD3 redirected T-cell targeting of NY-ESO-1- and LAGE-1-positive tumors. *Cancer Immunology, Immunotherapy.* **62** (4), 773-785, (2013).

7 Rongvaux, A. *et al.* Human hemato-lymphoid system mice: current use and future potential for medicine. *Annual Review of Immunology.* **31** 635-674, (2013).

8 Matsumura, T. *et al.* Functional CD5+ B cells develop predominantly in the spleen of NOD/SCID/gammac(null) (NOG) mice transplanted either with human umbilical cord blood, bone marrow, or mobilized peripheral blood CD34+ cells. *Experimental Hematology.* **31** (9), 789-797, (2003).

9 Zhang, T. *et al.* The binding of an anti-PD-1 antibody to FcgammaRIota has a profound impact on its biological functions. *Cancer Immunology, Immunotherapy.* **67** (7), 1079-1090, (2018).

10 Gamelli, R. L., Ershler, W. B., Hacker, M. P. & Foster, R. S. The effect of disulfiram on cyclophosphamide-mediated myeloid toxicity. *Cancer Chemotherapy and Pharmacology.* **16** (2), 153-155, (1986).

11 Dunay, I. R., Fuchs, A. & Sibley, L. D. Inflammatory monocytes but not neutrophils are necessary to control infection with Toxoplasma gondii in mice. *Infection and Immunity.* **78** (4), 1564-1570, (2010).

12 Ghasemlou, N., Chiu, I. M., Julien, J. P. & Woolf, C. J. CD11b+Ly6G- myeloid cells mediate mechanical inflammatory pain hypersensitivity. *Proceedings of the National Academy of Sciences of the United States of America.* **112** (49), E6808-6817, (2015).

13 Takao, K. & Miyakawa, T. Genomic responses in mouse models greatly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences of the United States of America.* **112** (4), 1167-1172, (2015).

14 Payne, K. J. & Crooks, G. M. Immune-cell lineage commitment: translation from mice to humans. *Immunity.* **26** (6), 674-677, (2007).

15 Mestas, J. & Hughes, C. C. Of mice and not men: differences between mouse and human immunology. *Journal of Immunology.* **172** (5), 2731-2738, (2004).

16 von Herrath, M. G. & Nepom, G. T. Lost in translation: barriers to implementing clinical immunotherapeutics for autoimmunity. *Journal of Experimental Medicine.* **202** (9), 1159-1162, (2005).

17 Mahdi, B. M. A glow of HLA typing in organ transplantation. *Clinical and Translational Medicine.* **2** (1), 6, (2013).

18 Shultz, L. D., Brehm, M. A., Garcia-Martinez, J. V. & Greiner, D. L. Humanized mice for

immune system investigation: progress, promise and challenges. *Nature Reviews Immunolog.* **12** (11), 786-798, (2012).

19 Brehm, M. A., Shultz, L. D., Luban, J. & Greiner, D. L. Overcoming current limitations in humanized mouse research. *Journal of Infectious Diseases.* **208 Suppl 2** S125-130, (2013).

20 Walsh, N. C. *et al.* Humanized Mouse Models of Clinical Disease. *Annual Review of Pathology.* **12** 187-215, (2017).

21 Shultz, L. D., Ishikawa, F. & Greiner, D. L. Humanized mice in translational biomedical research. *Nature Reviews Immunolog.* **7** (2), 118-130, (2007).

22 Brehm, M. A. *et al.* NOD-*scid* IL2rg^{null} (NSG) mice deficient in murine MHC Class I and Class II expression support engraftment of functional human T cells in the absence of acute xenogeneic GVHD following injection of PBMC. *The Journal of Immunology.* **200** (1 Supplement), 57.13-57.13, (2018).

23 King, M. *et al.* A new Hu-PBL model for the study of human islet alloreactivity based on NOD-scid mice bearing a targeted mutation in the IL-2 receptor gamma chain gene. *Clinical Immunology.* **126** (3), 303-314, (2008).

24 Ito, M. *et al.* NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood.* **100** (9), 3175-3182, (2002).

25 Shultz, L. D. *et al.* Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *Journal of Immunology.* **174** (10), 6477-6489, (2005).

26 Sasaki, E. *et al.* Development of a preclinical humanized mouse model to evaluate acute toxicity of an influenza vaccine. *Oncotarget.* **9** (40), 25751-25763, (2018).

27 Tobin, L. M., Healy, M. E., English, K. & Mahon, B. P. Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. *Clinical and Experimental Immunology.* **172** (2), 333-348, (2013).

Figure 1

Click here to access/download;Figure;Figure 1 Myeloablation of mice using cyclophosphamide.tif

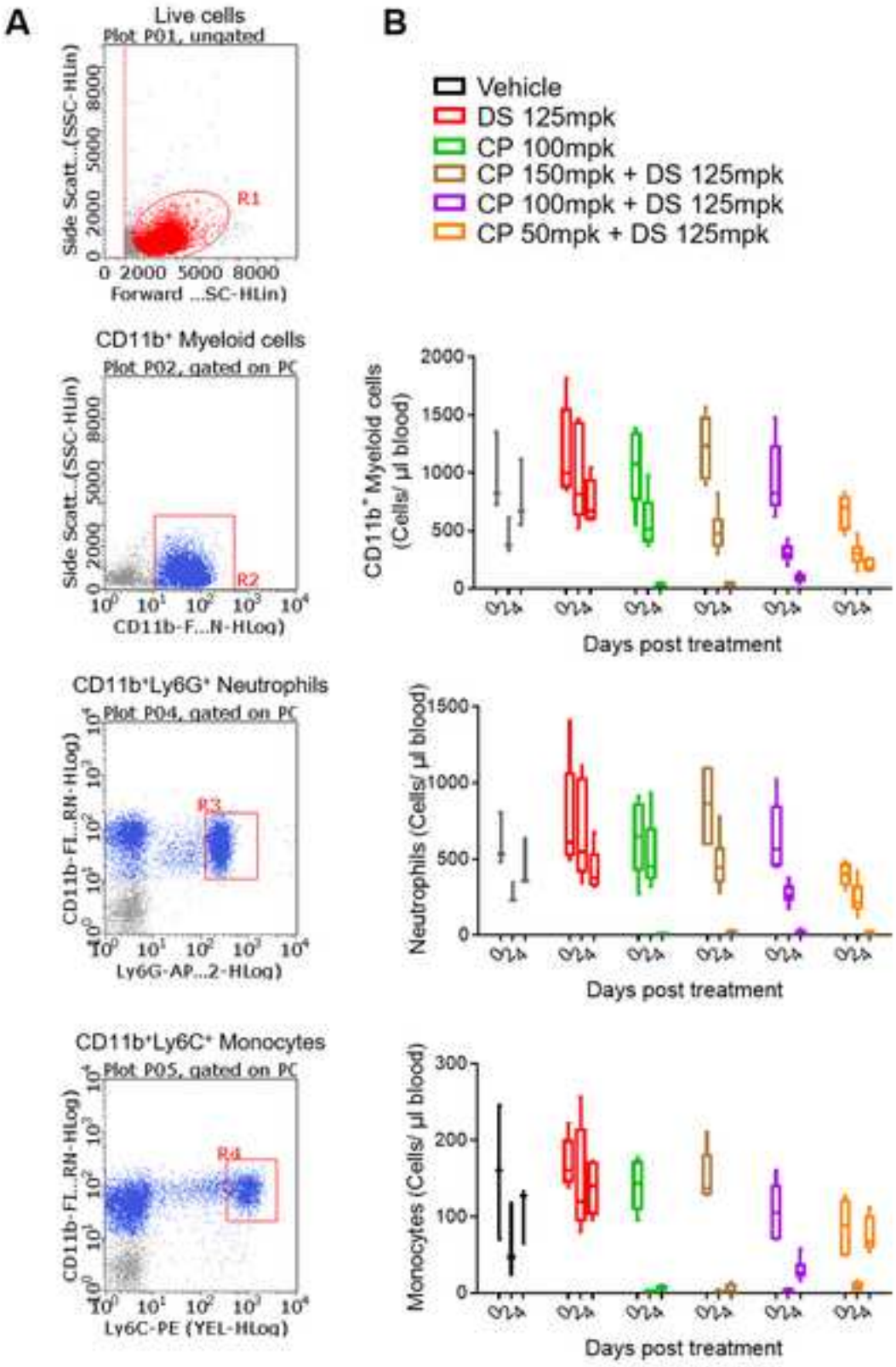


Figure 2

Click here to access/download;Figure;Figure 2 Human PBMC transplantation and tumor engraftment.tif

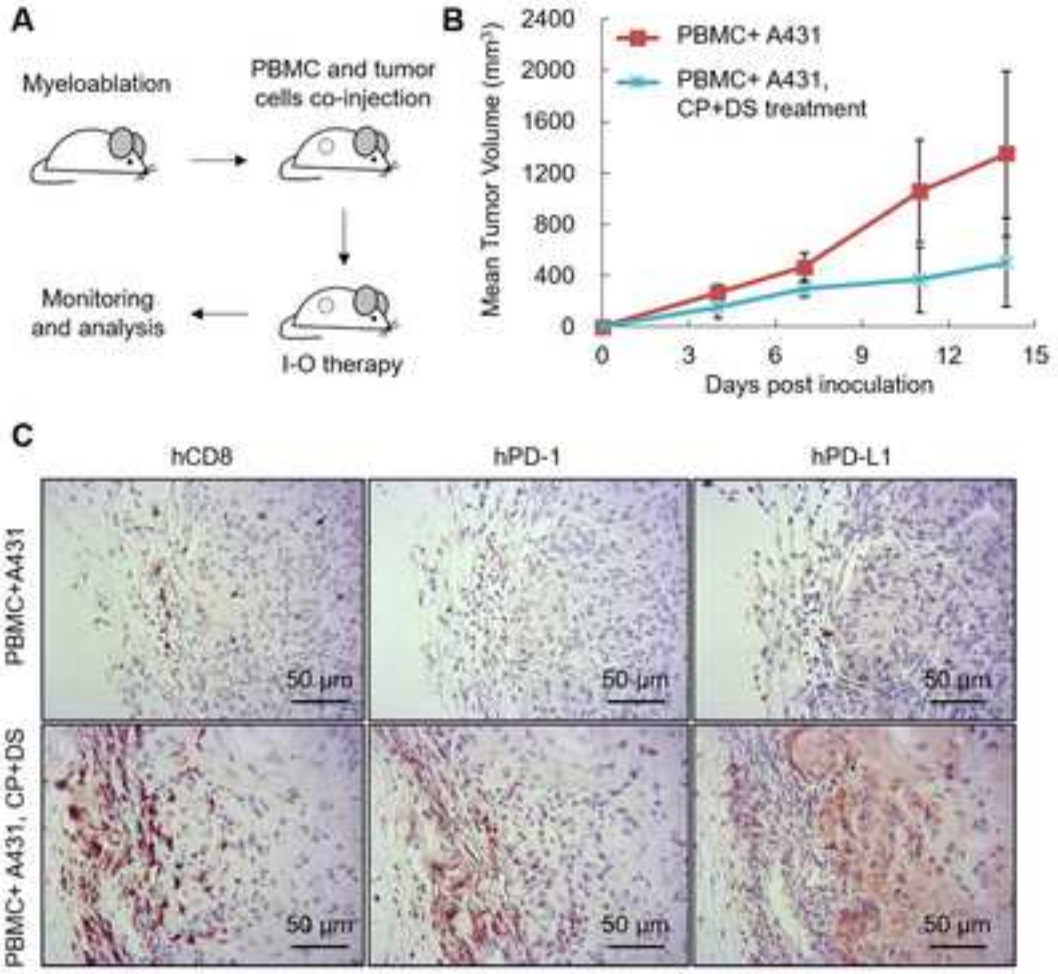


Figure 3

Click here to access/download;Figure;Figure 3 PBMC donor and human cancer cell line screen.tif

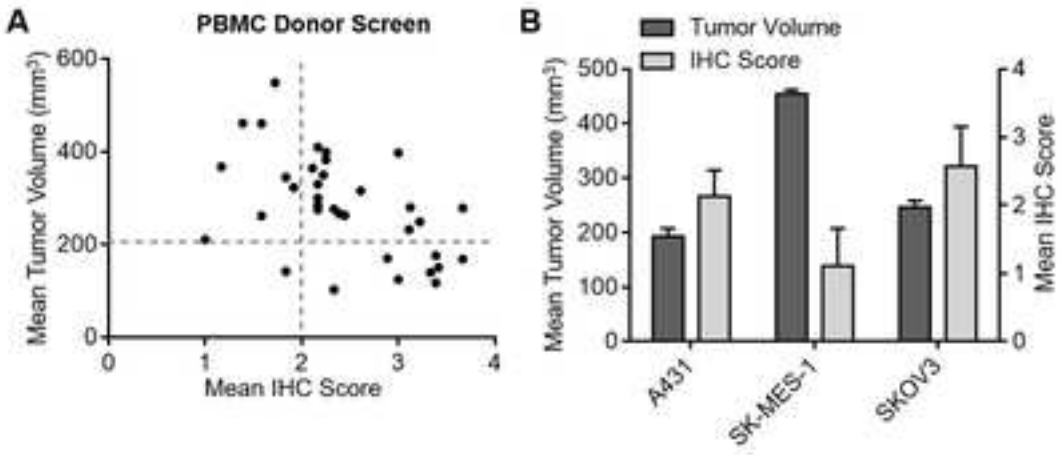
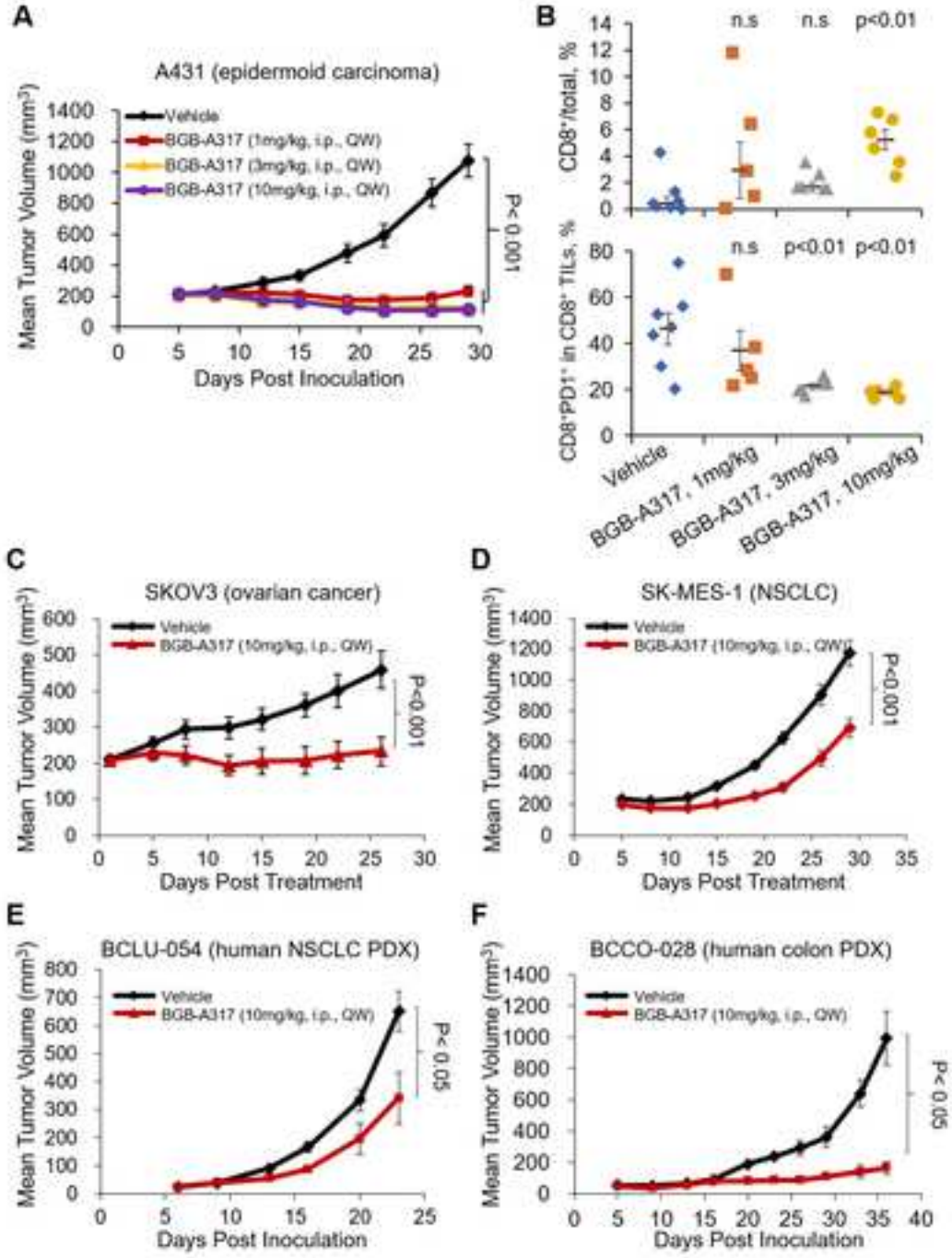


Figure 4

Click here to access/download;Figure;Figure 4 Anti-tumor activities and pharmacodynamics analysis.tif



Name of Material/ Equipment	Company
PBMC separation /cell culture	
Histopaque-1077	Sigma
DMEM	Corning
DPBS	Corning
FBS	Corning
Penicillin-Streptomycin, Liquid	Gibco
Trypsin-EDTA (0.25%), phenol red	Gibco
Matrigel	Corning
FACS analysis	
Deoxyribonuclease I from bovine pancreas	Sigma
Collagenase Type I	Sigma
Anti-mouse/human CD11b (M1/70) antibody	BioLegend
Anti-mouse Ly-6C (HK1.4) antibody	BioLegend
Anti-mouse Ly-6G (1A8) antibody	BioLegend
Anti-human CD8 (OKT8) antibody	Sungene Biotech
Anti-human CD279 (MIH4) antibody	eBioscience
Anti-human CD3 (HIT3a) antibody	4A Biotech
Guava easyCyte 8HT Benchtop Flow Cytometer	Millipore
Tumor/PDX implantation /dosing / measurement	
Cyclophosphamide	J&K
Disulfiram	J&K
Syringe	BD
Hypodermic needles (14G)	Shanghai SA Mediciall & Plastic Instruments Co., Ltd.
Vernier Caliper (MarCal)	Mahr
IVC individual ventilated cages	Lingyunboji Ltd.
IHC	
Leica ASP200 Vacuum tissue processor	Leica
Leica RM2235 Manual Rotary Microtome for Routine Sectioning	Leica
Leica EG1150 H Heated Paraffin Embedding Module	Leica
Ariol-Clinical IHC and FISH Scanner	Leica
Anti-human CD8 (EP334) antibody	ZSGB-Bio

Anti-human PD1 [NAT105] antibody	Abcam
Anti-human PD-L1 (E1L3N) antibody	Cell Signaling Technology
Polink-2 plus Polymer HRP Detection System	ZSGB-Bio

Catalog Number	Comments/Description
10771	Cell isolation
10-013-CVR	Cell culture
21-031-CVR	Cell culture
35-076-CV	Cell culture
15140-163	Cell culture
25200-114	Cell culture
356237	CDX inoculation
DN25	Sample preparation
C0130	Sample preparation
101206	FACS
128008	FACS
127614	FACS
H10082-11H	FACS
12-9969-42	FACS
--	FACS
0500-4008	FACS
Cat#419656, CAS#6055-19-2	In vivo efficacy
Cat#591123, CAS#97-77-8	In vivo efficacy
300841	CDX inoculation
0.7*32 TW SB	PDX inoculation
16ER	Tumor measurement
IVC-128	Animal facility
ASP200	IHC
RM2235	IHC
EG1150 H	IHC
Ariol	IHC
ZA-0508	IHC

ab52587	IHC
13684S	IHC
PV-9001/9002	IHC

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Author(s): Zhiyu Li, Xiao Yang, Yilu Zhang, Xiaolong Yang, Yanjuan Zhang, Wenfang Gong, Huichen Bai, Ning Liu, Xinxin Cui, Zhiyu Tang, Mingming Guo, Kang Li, Tong Zhang, Lei Wan, Xiaomin Song

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Institution:	Beigene
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Signature:	Xiaomin Song
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DETAILED RESPONSE TO THE REVIEWERS

Reviewer #1

Manuscript Summary:

Li et al report a humanized mouse model for the reconstitution of human tumors and human lymphocytes for the evaluation of immune checkpoint inhibitors in cancer immunotherapy. Their model consists of administering cyclophosphamide to NOD-SCID mice so that they accept human tumors infiltrated with PBMC. Whilst the data they present suggests that the model is effective for evaluating the human CD8 T cell response in an immunodeficient mouse model it is not novel as there are several models of human T cell reconstitution in immunodeficient mice and numerous studies of human tumor biology and cell growth in such models as well.

We thank Reviewer 1 for acknowledging that our model is useful for the evaluation of immune checkpoint inhibitors in cancer immunotherapy. We have taken good note of the issues raised by the reviewer regarding the novelty of our models. To this end, we provide additional data and literature analysis that, in our opinion, have strengthened the novelty of our humanized mouse models. In brief, our models provide partially reconstituted human immunity in human tumor bearing mice through subcutaneously admixing of human PBMC with cancer xenografts. This approach provides a rapid and cost-effective, yet highly reproducible alternative to full stem cell reconstitution (e.g. CD34+ hematopoietic stem cell-engrafted humanized mice). Our model has been proved to be useful for evaluating T cell-engaging cancer immunotherapies, particularly when working on short timelines or to select agents before moving to a more complex multi-lineage immunity model.

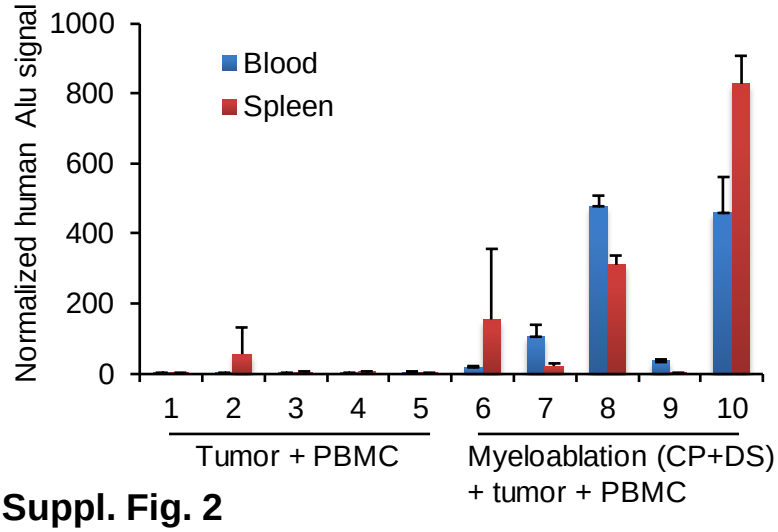
Major Concerns:
Lack of novelty. Similar models have been described previously. There are several issues, if clarified, would improve the manuscript and clarify issues for the reader:

We thank Reviewer #1 for the suggestions. We have clarified these issues in our revised manuscript, and please also refer to the following point-to-point responses.

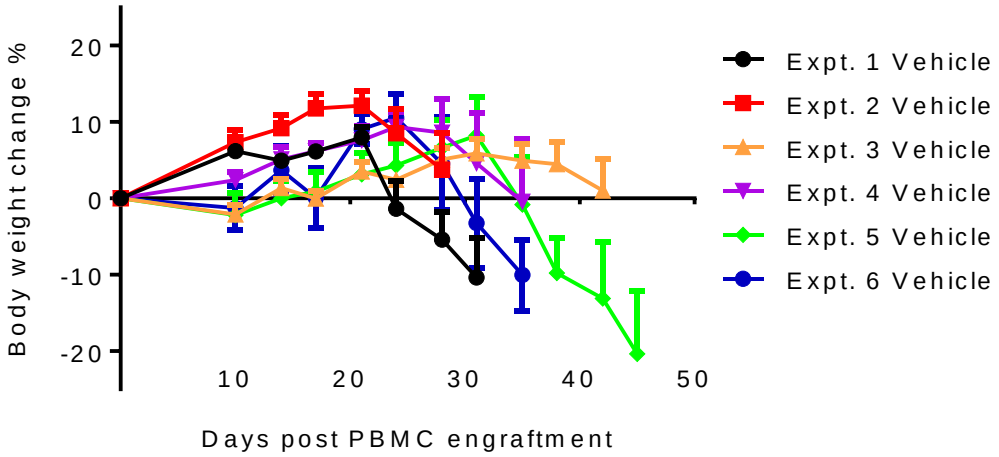
*The authors infiltrate the tumor with human PBMC. However, they only provide evidence for CD8Tcell infiltration. This raises several questions: is there human Immune cell reconstitution within the NOD-SCID mice or do the cells only reside in the tumor. If so, what cells are reconstituted? Is is only CD8 T cells? Is there reconstitution of non-T cells such as B cells?

The authors do not have evidence suggesting the reconstitution of human immune cell populations other than CD8+ T cells. We do believe, however, there's systemic presence of human immune cells, as suggested by at least two lines of evidence: 1) Cyclophosphamide pre-treated, PBMC-engrafted mice demonstrate significant elevation of human Alu sequence signal in the blood and spleen compared to the control mice, quantified by RT-PCR at 14 days post subcutaneous PBMC injection (Suppl. Fig. 1). 2) Clinical observations associated with xenogeneic GVHD in PBMC-engrafted mice, about 30 days post engraftment (Suppl. Fig. 2, and as detailed below).

Suppl. Fig. 1



Suppl. Fig. 2



*Related to this question: do the mice develop xenogeneic GVHD? *If not how long do the human cells survive in this model as human T cell reconstituted in NOD SCID mice are usually short lived?

Clinical observations commonly associated with xenogeneic GVHD (xGVHD) have been observed in our humanized model, such as erythema, hunched posture, weight loss (Suppl. Fig. 2) and mortality. These phenotypes were usually observed towards the end of our studies, usually at 1-2 months post engraftment, indicating the propagation and infiltration of human T cells in xGVHD target organs. The prior treatment of CP+DS increase the engraftment of human PBMCs. In tumor tissues, our immunohistochemistry (Fig. 2C) and flow cytometry (Fig. 4B) analyses demonstrated the presence of human CD8+ T cells at least 20-25 days post PBMC engraftment.

*Have the authors been able to separate xenogeneic GVHD, allo-immunity, and reconstitution of mouse macrophages from the anti-tumor effect.

We thank Reviewer #1 for raising this question. Alloimmunity describes the immune interaction between genetically distinct individuals, and this type of immune response is expected in our experimental models as the human cancer cell lines or PDX transplant is a foreign substance to

the donor PBMC. The authors believe that the enhanced anti-tumor activities of tested immunotherapy are largely due to T cell potentiation within the scope of alloimmunity. Xenogeneic GVHD (xGVHD), as discussed above, did occur in our humanized mouse models but at a much later stage than when the anti-tumor effects being observed, suggesting that the anti-tumor efficacies are largely due to alloimmunity but not xGVHD. In addition, our previous study has suggested that presence of tumor-associated macrophages (mCD64+) negatively correlate with anti-PD-1 antibody-mediated anti-tumor activities[1].

Minor Concerns:
*It is not clear why they authors used NOD-SCID mice rather than NSG mice which are more accommodating of human immune cells.

The authors acknowledge that the more severely immune-compromised NSG mice could be a better model for human immune cell engraftment. In fact, our in-house data support that the protocol documented in this manuscript shall work for both NOD-SCID and NSG mice. The authors started the relevant projects a few years ago, when NSG mice are not yet very popular and readily available. We performed our model establishment and proof-of-concept experiments using the NOD-SCID strain, and in order to keep our data consistent, we continued using this mouse strain for the later studies. Furthermore, the higher costs associated with using the NSG strain draw our preference to the NOD-SCID strain as, to our experience, there was no significant benefit in using the more immuno-compromised NSG strain.

In summary, more information is required surrounding the degree of immune reconstitution, the mechanism of human-immune antitumor response, and the limitations of the model.

We thank and agree with Reviewer #1 and have provided additional discussions regarding the above-mentioned issues in our revised manuscript.

Reviewer #2

Manuscript Summary:

In this study, the authors developed the humanized mice model that human PBMC and tumor cells were subcutaneously inoculated to investigate the anti-tumor activity of immune checkpoint inhibitors. Although the idea is not bad, some figures are unclear for publication.

We thank Reviewer #2 for acknowledging the interesting idea of our manuscript. We have revised some of our figures (Fig. 1 in particular) that might cause confusion. Please see the details below.

Major Concerns:

In Fig. 1A, the author described gating strategy of myeloid cells, but it looks very strange that almost myeloid cells are PMN-MDSC or M-MDCS population and neutrophils are very low frequency, even you did not show % of those fractions. Generally, CD11b+ myeloid cells composed 70-80% of Gr1+ granulocytes and 10-20% F4-80+ monocytes in peripheral blood from NOD-scid, NSG, or NOG mice, and HLADR negative MDSC is very rare population in steady state. Also, the authors referred Ali, K. et al. Nature. 510 (7505), 407-411, for creating the gates. In this reference, they showed the PMN-MDSC or M-MDCS population by Ly6G/Ly6C/CD11b staining, but those cells are in splenocytes but not in peripheral blood as you mentioned in the figure. The reviewer think that is critical error to defining the cell subsets, MDSCs and neutrophil populations in PB. In Fig. 1B, there is also severe concern about the frequency of neutrophils in PB, only 1-2 % is too small even though cyclophosphamide was not treated.

The authors thank the reviewer for pointing out our inappropriately defined cell populations in the mouse peripheral blood. MDSCs, identified as CD11b+Ly6G/Ly6C+ subsets, are differentiated from the myeloid lineage under inflammatory conditions or cancers and infiltrated into inflammation sites and tumors, and should not be used in this case. The authors acknowledge that we have incorrectly characterized populations in the peripheral blood. In the blood, Ly6C is believed to be a marker of monocytes ($Ly6C^{high}$) and neutrophils ($Ly6C^{interm}$), and $Ly6G^{+}$ subsets are characterized as neutrophils[2,3]. We have therefore re-analyzed our flow cytometry data accordingly. In this case, the neutrophil population ($CD11b^{+}Ly6G^{+}$) accounts for about 60%, and the monocyte population ($CD11b^{+}Ly6C^{high}$) accounts for 10-20% of the $CD11b^{+}$ myeloid cells. Figure 1 has been revised accordingly.

The authors should describe about the purpose/necessity to utilize the disulfiram, but it looks no effect.

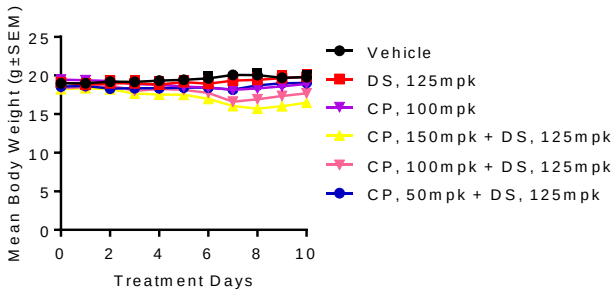
Disulfiram (DS) decreases the urotoxicity of cyclophosphamide (CP) in mice, and CP combined with DS has been suggested to have longer-lasting neutropenia than animals treated with CP alone[4]. We have explanined our rationale of utilizing disulfiram in our revised protocol section 1.1.3. Our own data also suggest that CP+DS would reduce death incidence compared to the CP-only treated group (Suppl. Fig. 3).

How many mice did you use in the experiment?

6 mice were used in our myeloablation dose determination experiments (protocol section 1.1), 3 mice per PBMC donor were used in our PBMC donor screen (protocol section 2) , 3 mice per group were used in our cancer cell line and PDX screen (protocol section 3), and 8-12 mice per group were used in our efficacy studies (protocol section 5). We have included the number of mice used for each study in our revised manuscript.

Suppl. Fig. 3

Group	Mortality
Vehicle	0 (of 3)
DS, 125mpk	0 (of 6)
CP, 100mpk	5 (of 6)
CP, 150mpk + DS, 125mpk	5 (of 6)
CP, 100mpk + DS, 125mpk	0 (of 6)
CP, 50mpk + DS, 125mpk	1 (of 6)



Minor Concerns:

1) The author should describe about more detailed information of each tumor cell line/PDXs and the protocol for the treatment of BGB-A317.

We thank the reviewer for pointing this out and we have elaborated our BGB-A317 treatment protocol and cell lines/ PDXs used in the protocol section 5.4.

2) The author should explain the objective criteria of the PD-1, PD-L1, and CD8 IHC scoring.

We thank the reviewer for pointing this out and we have included our IHC scoring system in our revised protocol (section 4). Please also see the description below:

Human CD8 and PD-1 expression on tumor-infiltrating leucocytes (TIL) were assessed by assigning an expression score on a 5-point scale (IHC score, range 0–4) within the selected core area at high magnification (20x, 40x). 0, absent; 1, weak intensity/ less than 20% cells; 2, weak-to-moderate intensity/ 20%–50% cells; 3, moderate-to-strong intensity/ 50%–80% cells; 4, strong intensity/ more than 80% cells. Human PD-L1 staining within tumor cells was scored on a 5-point scale (IHC score, range 0–4). 0, absent; 1, weak intensity/ less than 10% cells; 2, weak-to-moderate intensity/10%–30% cells; 3, moderate/ 30%–50% cells; 4, strong intensity/ more than 50% cells.

3) The reviewer would like to know the ratio of CD4 and CD8 in CD3+ T cells in TIL, and did CD8 level increase after 10 mg/ml BGB treatment?

The authors do not have relevant data for CD4+ T cells and thus not able to calculate the ratio of CD4+ and CD8+ in the CD3+ TILs. The percentage of CD8+ TILs does increase after 10mg/kg BGB-A317 treatment, compared to the vehicle group (as shown in Fig. 4B).

Reviewer #3

Reviewer comments

In the manuscript entitled "A Human Peripheral Blood Mononuclear Cell (PBMC) Engrafted Humanized Xenograft Model for Translational Immuno-oncology (I-O) Research" Zhuo Li and colleagues describe a protocol for generating humanised mice, harbouring human immune cells in addition to human tumours. The human tumours are established by subcutaneous injection of human tumour cell lines or transplantation of PDX tumour tissue. The field of immuno-oncology is rapidly developing and there is need for reliable preclinical models overcoming the trans-species limitations associated with use of immunocompetent mouse models in immuno-oncology preclinical studies. Thus, development of humanised pre-clinical tumour models is highly relevant.

The manuscript describes a method that might be used by other scientist in the field. The need for clarity in each step is high, thus the authors have to clarify some of the steps in the described protocol. In addition, additional control experiments are required to establish this model as relevant for translational immuno-oncology research.

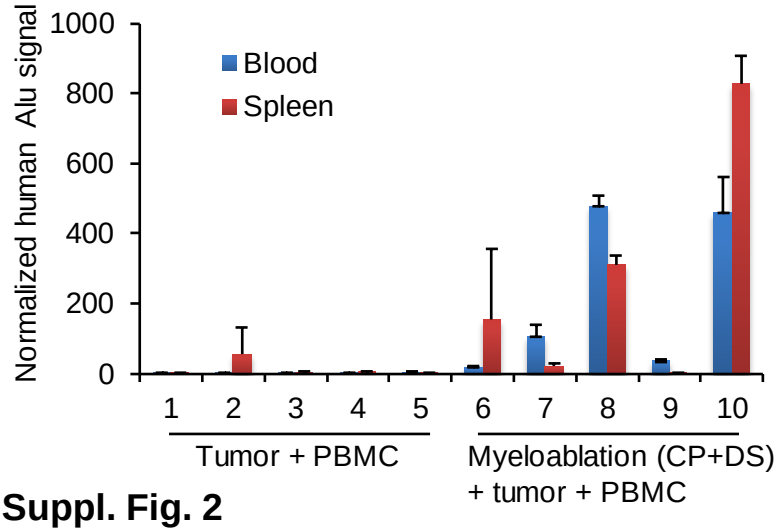
We thank reviewer #3 for highlighting the importance of our work that aims to establish reliable humanized preclinical tumor models for translational immuno-oncology studies. We have included additional information and details for our model development in our revised manuscript.

Major concerns

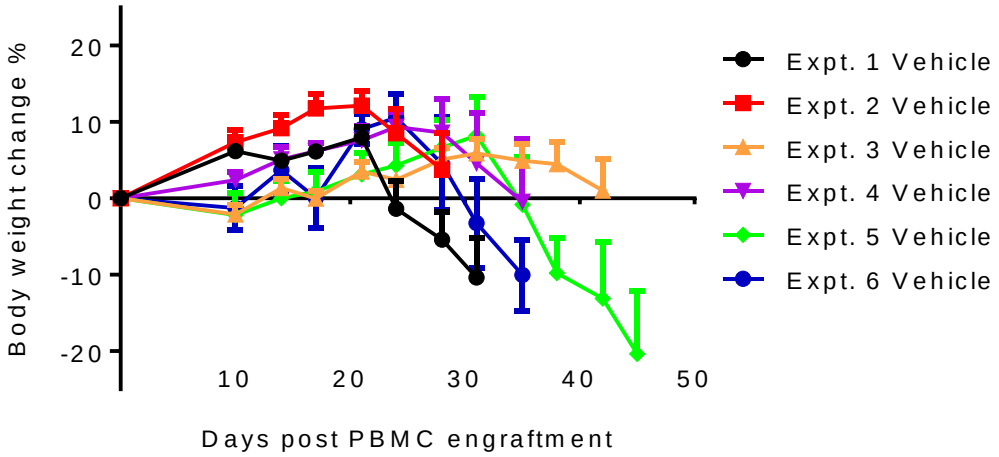
- In the described protocol PBMCs are injected subcutaneously together with human cancer cell lines in a 50% Matrigel suspension. The authors have not provided any data describing a potential systemic presences of human immune cells, expected to be mainly T cells, in the transplanted mice. Flow cytometry analyses aiming at detecting human immune cells in blood collected from the humanised mice, at different time points, should be provided, as well as immunohistochemistry (IHC) or flow cytometry analyses of other organs that might harbour the human immune cells, mainly spleen.

Although human immune cells in the blood or other organs were not tested in a dynamic manner and profiled by IHC or flow cytometry, we do believe, however, there's systemic presence of human immune cells, as suggested by at least two lines of evidence1) Cyclophosphamide pre-treated, PBMC-engrafted mice demonstrate significant elevation of human Alu sequence signal in the blood and spleen compared to the control mice, quantified by RT-PCR at 14 days post subcutaneous PBMC injection (Suppl. Fig. 1). 2) Clinical observations commonly associated with xenogeneic GVHD have been observed in our humanized model, such as erythema, hunched posture, weight loss (Suppl. Fig. 2) and mortality. In addition, we have toned down some of our statements as these might have over-interpreted the degree of humanization of our mouse models.

Suppl. Fig. 1



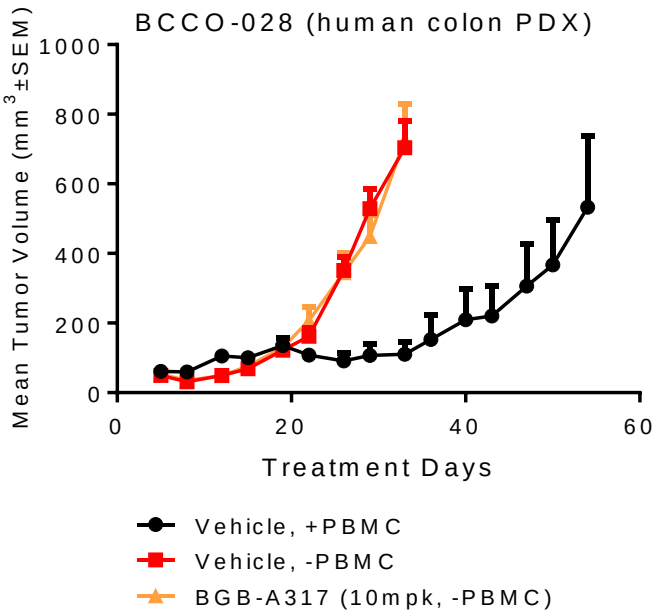
Suppl. Fig. 2



- The authors should show data comparing tumour growth in the presence and absence of PBMCs, for the studied cell lines and PDX models. The manuscript lacks sufficient controls to state that the observed effect of BGB-A317 treatment is dependent PBMC mediated humanisation. Non-humanised/mock-humanised mice should be treated with BGB-A317 under the same conditions as in the described experiments (Figure 4), to validate that the observed effect of BGB-A317 treatment is dependent on the presence of human immune cells.

The tumor growth curve of one of the studied tumor models, human colon PDX BCCO-028, in the presence and absence of PBMC is provided for the reviewer's reference (Suppl. Fig. 4). Although this data is collected in a separate experiment from our Fig. 4F, it is clear that there is no anti-tumor effects of BGB-A317 without PBMC humanization. The authors also noted that similar information of non-humanized/ mock-humanized mice was not shown in many published literatures involving models with allogenic PBMCs or peripheral blood lymphocytes (PBLs)[5,6]. We believe that the PBMC-engrafted, BGB-A317 non-treated group (i.e. vehicle groups in Fig. 4) that were included in all our studies, served as a valid control for evaluating the anti-tumor activities of the tested immunotherapies.

Suppl. Fig. 4



- The authors should refer to all figures at the appropriate place in the text.

We apologize for not referring to the figures at the appropriate locations in the text. We have revised our manuscript following the journal's requirements.

- In the discussion the authors elaborate on graft versus host disease (GvHD) as a limiting factor to this protocol. A note about the expected experimental window should be provided in the protocol.

We thank reviewer #3 for pointing this out. We elaborated on this issue in the protocol 5.5 and discussion sections of our revised manuscript.

Minor comments

- The license number for the ethical approval for obtaining the informed consents should be provided.

We have attached a copy of our informed consent form for voluntary blood donation used in our studies. We ensure that all participants understand the scope of our research, their involvement and the rights and risks while involved in research. This informed consent form was signed by each donor everytime of the studies and the original copies have been properly stored in our laboratory archive.

- All abbreviations should be explained first time in use.

We thank the reviewer for pointing this out. We have proofread our manuscript and ensure all abbreviations properly explained the first time used.

- The authors should comment on whether only female mice can be used for this procedure or if male mice also are suitable. Alternatively, if only female mice have been investigated, this should be noted.

All mice involved in this study were female. The authors have not evaluated if there is difference between male and female mice. We thank the reviewer for pointing this out and we have clarified this in section 1.1.1 in the revised manuscript.

- Section 1.1.2: "Prepare cyclophosphamide (CP, CTX) at different doses (50, 100 and 150 mg/kg, J&K) in saline". The authors should explain why different solutions are prepared. Were different solutions prepared to enable administration of an equal volumes of drug solution to mice getting different doses of cyclophosphamide?

We thank reviewer for pointing this out and the reviewer's understanding is correct. We have clarified this in section 1.1.2 in the revised manuscript.

- Section 1.1.4: When is the blood on day 0 collected, before or after administration of cyclophosphamide and disulfiram?

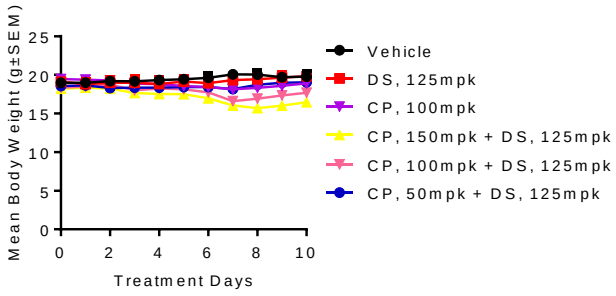
The blood is collected on day 0 before the administration of cyclophosphamide and disulfiram. We have clarified this in section 1.1.4 in the revised manuscript.

- Section 1.1.6: The authors should provide body mass development curves for treated and non-treated mice.

The body weight development curves for treated and non-treated mice is provided for the reviewer's reference (Suppl. Fig. 3).

Suppl. Fig. 3

Group	Mortality
Vehicle	0 (of 3)
DS, 125mpk	0 (of 6)
CP, 100mpk	5 (of 6)
CP, 150mpk + DS, 125mpk	5 (of 6)
CP, 100mpk + DS, 125mpk	0 (of 6)
CP, 50mpk + DS, 125mpk	1 (of 6)



- Section 1.2.3: The authors should state the number of hours after last administration of CP rather than "the day after".

We thank the reviewer for pointing this out and we have clarified this in section 1.2.3 in the revised manuscript.

- Section 1.2.4: For how many days/weeks were the measurements recorded?

We thank the reviewer for pointing this out and we have clarified this in section 1.2.4 in the revised manuscript.

- Section 2 and Figure 3: How many healthy PBMC donors were screened?

We thank the reviewer for pointing this out and we have clarified this in section 2.1 in the revised manuscript. The authors have screened over 50 healthy PBMC donors in the study, to ensure we have enough number of suitable donors. The authors would also like to note that any searcher who want to adopt this protocol might decide on their own how many healthy PBMC donors to be screened, based on the size of their planned studies.

- Section 5 and Figure 3: The amount of administered PBMCs is referred to as a range, 2.5-5x10⁶ PBMCs. This difference in the administered amount of PBMCs might influence the degree of humanisation and tumour growth. The authors should specify the amount of PBMCs administered to each mouse. If the authors have data supporting that there are no differences in degree of humanisation or tumour growth between administration of 2.5 and 5 x10⁶ PBMCs, these data are very interesting and should be shown.

The authors apologize for this confusion. The number of PBMC used for any individual mouse in one single study is the same. However, due to variations in the availability of total isolated PBMC at the time of each study, the authors have chosen to use 2.5 x 10^6, 4 x 10^6, or 5x10^6 PBMC per mouse. Although this 2-fold difference in the administered amount of PBMCs might affect the degree of humanization, the authors do not observe significant differences in evaluating anti-tumor efficacies of the tested immunotherapies. We have not performed a detailed side-by-side comparison examining the different number of PBMC used and the degree of humanization. We thank the reviewer for pointing this out and we have clarified this in section 5.2 in the revised manuscript.

- Section 5.3: Were the PBMCs injected on each side of the engrafted piece of PDX tumour tissue administered in a Matrigel solution, as described for the cell lines? If the PBMCs were administered without Matrigel, did this influence the level of human immune cells in the blood compared to administration of PBMCs in Matrigel?

PDX tumor tissues were administered in a Matrigel solution, same as described for the cell lines. We thank the reviewer for pointing this out and we have clarified this in section 5.3 in the revised manuscript. We have not performed a detailed side-by-side comparison examining the influence of using or not using Matrigel on the systemic engrafment of PBMC.

- Section 5.7: For how long were the cells incubated with the antibodies at 4°C?

We thank the reviewer for pointing this out and we have detailed the duration of antbody incubation in section 5.7 in the revised manuscript.

- Figure 1: The authors should explain the statistics used and give values for *, ** and ***.

We thank the reviewer for pointing this out and we have clarified this in the revised manuscript.

- Figure 1B: How many mice were included in each group?

We thank the reviewer for pointing this out and we have clarified this in the revised manuscript.

- Figure 2: The authors should clarify what is meant by "No treatment" and "Cyclophosphamide"/"+ Cyclophosphamide". Is "No treatment" in the absence of cyclophosphamide or PBMCs? Were the "Cyclophosphamide"/"+ Cyclophosphamide" groups also treated with disulfiram, or is this experiment equal to the red coloured group in figure 1B?

The authors apologize for this confusion. We thank the reviewer for pointing this out and we have clarified this in legend of Fig. 2B in the revised manuscript. The red line represents data from the group with PBMC and tumor transplation but without CP or disulfiram treatment (supposedly poor humanization), while the blue line represent data from mice with PBMC+tumor+CP+disulfiram.

- Figure 2C: Scale bars should be included in the images.

We thank the reviewer for pointing this out and we have included the scale bar in our revised manuscript (Fig. 2C)

- Figure 3: The authors should clarify the amount of PBMCs administered to the mice (see previous comment).

Please refer to the response above. In brief, the number of PBMC used for any individual mouse in one single study is the same. However, due to variations in the availability of total isolated PBMC at the time of each study, the authors might choose to use 2.5 x 10^6, 4 x 10^6, or 5x10^6 PBMC per mouse at different studies.

- Figure 3A: Does each dot represent the mean value of 3 mice engrafted with PBMCs from 1 donor, in combination with A431 cells? How many PBMC donors were screened?

It is correct that each dot represents the mean value of 3 mice engrafted with PBMCs from 1 donor, in combination with A431 cells. We thank the reviewer for pointing this out and we have clarified this in legend of Fig. 3A in the revised manuscript. The authors have screened over 50 healthy PBMC donors in the study, and the results shown in Fig. 3A are representative data from 35 of them.

- Figure 3B: How many mice are included in each group? Please provide a short description of the scoring system 0-4?

3 mice were included in each group. We thank the reviewer for pointing this out and we have clarified this in legend of Fig. 3B in the revised manuscript.

We have included our IHC scoring system in our revised protocol (section 4). Please also see the description below:

Immunohistochemical scoring was performed in a blinded fashion by an experienced pathologist (M. Guo). Immuno-stained sections were initially assessed at low magnification (4x, 10x) to select the core with the highest density of positive cells. TIL markers (indicating immune cell subsets) and human PD-L1 (expressed by tumor cells) were assessed. The area of the entire core occupied by tumor epithelium versus stroma was assessed followed by estimation of the proportion of positive TILs that were intraepithelial or intrastromal (intraepithelial localization defined as lymphocytes within tumor cell nests and/or adjacent to and in direct contact with tumor cells).

Human CD8 and PD-1 expression on tumor-infiltrating leucocytes (TIL) were assessed by assigning an expression score on a 5-point scale (IHC score, range 0–4) within the selected core area at high magnification (20x, 40x). 0, absent; 1, weak intensity/ less than 20% cells; 2, weak-to-moderate intensity/ 20%–50% cells; 3, moderate-to-strong intensity/ 50%–80% cells; 4, strong intensity/ more than 80% cells. Human PD-L1 staining within tumor cells was scored on a 5-point scale (IHC score, range 0–4). 0, absent; 1, weak intensity/ less than 10% cells; 2, weak-to-moderate intensity/10%–30% cells; 3, moderate/ 30%–50% cells; 4, strong intensity/ more than 50% cells.

- Figure 4: Please, state in the figure legend how many PBMCs that were administrated? Control experiments including non-humanised mice transplanted with the various cancer models and treated with BGB-A317 must be included (see previous comment).

We have included the amount of PBMC used in each study in the revised manuscript. Please refer to our response above regarding the control experiments using non-humanzied mice.

Reviewer #4

Summary:

Although many murine immuno-oncology (IO) models have been created they possess well documented limitations in their ability to recapitulate a human immune system. The authors present data in support of using humanized mice for to evaluate potential I-O agents in the context of a human immune response. The use of humanized mice in I-O studies is limited by the considerable cost, screening human donors and time required to generate the mice. The proposed model seeks to address the time required to generate a relevant model by incorporating a myeloablation strategy (combined treatment with cyclophosphamide and disulfiram) prior to engrafting human PBMC from healthy donors in NOD/SCID mice. The authors provide evidence that tumor control can be improved in mice receiving BGB-A317 (Tislelizumab), an anti-PD1 antibody, as a proof of concept for using a humanized PBMC model for evaluation of I-O agents.

By creating their humanized PBMC xenograft model, the authors convincingly highlight the importance of such a model to study human immune responses and human cancers in translational I-O. This reproducible and robust in vivo model has the potential to be used for various I-O therapy studies.

We thank Reviewer 4 for her/his overall positive comments and constructive suggestions. We also thank Reviewer 4 for highlighting the importance of our PBMC-reconstituted humanized mouse model to be potentially used for various I-O therapy studies.

Major Criticisms:

1. Most of the literature on humanized PBMC models (Sasaki 2018, Weibmuller 2015, Tobin 2013, Lin 2018) use either IV injection for short-term studies or IP for long-term studies. This should be highlighted somewhere in the discussion. Furthermore, what would be the risks and benefits of using this novel method of injecting PBMCs admixed with tumor cells subcutaneously (in matrigel) versus previously published studies or IV or IP? For example, PBMC contain functional NK cells and it is unclear what contribution other immune cell components play in the progression of the tumor. In this model it would appear that true engraftment does not happen but intra-tumoural human immune cells can be added to the TME with this technique. What is the advantages of this are over a simple PDX model where small fragments of whole tumour (with intact TME) are being implanted into NOD/SCID mice?

The authors thank Reviewer 4 for the suggestion of highlighting the fact that most humanized PBMC models have chosen IV or IP as the route of injection[7,8]. To this end, we discussed the possible advantages and disadvantages of our model in the revised manuscript. We have also toned down some of our statements as these might have over-interpreted the degree of humanization of our mouse models.

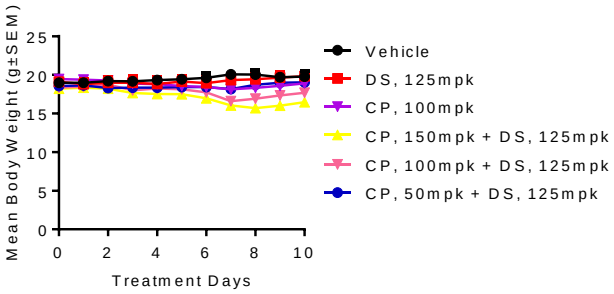
We thank Reviewer 4 for pointing out the comparison between a simple PDX model versus our humanized model through co-engraftment of human PBMC. Patient-derived tumor immune microenvironments co-exist in early passages of PDXs, but the stromal tissues, including immune cells, diverge substantially from their parental tumors and decrease significantly during passaging[9,10]. With the limitation on available tissues for implantation and possibly low engrafment rate, usually this is not applicable for experiments requiring relatively large animal numbers. Therefore, humanization of the immuno-compromised mice by co-engraftment, along with the tumor, of normal human PBMC would circumvent some of these issues. The reconstitution of the human immune system would therefore allow examination of the role of interactions between xenogeneic human stroma and tumors in cancer progression.

2. Myeloablation and figure 1B: the ablation with CP and DS is shown in Figure 1B. However, there is no mention of the main advantages of adjuvant chemical ablation in the NOS/SCID mice versus radiation ablation. Previous studies have shown reliable response for humanized mice when using radio-ablation. A rationale for choosing CP+DS should also be provided given that single agent CP treatment alone appears to reduce Neutrophils to a similar extent.

We thank the reviewer for pointing out the issue regarding the rationale of using adjuvant chemical ablation. As the reviewer pointed out, there are many studies which used the radio-ablation strategy. The authors do not think, however, there will be a significant difference between these two myeloablation approaches, and have some preliminary in-house experience suggesting that both methods work well in our models (data not shown). Disulfiram (DS) decreases the urotoxicity of cyclophosphamide (CP) in mice, and CP combined with DS has been suggested to have longer-lasting neutropenia than animals treated with CP alone[4]. We have explanined our rationale of utilizing disulfiram in our protocol section 1.1.3. Our own data also suggest that CP+DS would reduce death incidence compared to the CP-only treated group (Suppl. Fig. 3). One possible advantage of the adjuvant chemical ablation over radio-ablation is that this would be more applicable to research labs who have limited access to radiation instruments.

Suppl. Fig. 3

Group	Mortality
Vehicle	0 (of 3)
DS, 125mpk	0 (of 6)
CP, 100mpk	5 (of 6)
CP, 150mpk + DS, 125mpk	5 (of 6)
CP, 100mpk + DS, 125mpk	0 (of 6)
CP, 50mpk + DS, 125mpk	1 (of 6)



Furthermore, no information is provided on the number of animals making it difficult to interpret the variability in responses to CP+DS ablation and raises a strong concern for reproducibility if adopted by other labs. In addition, no data is provided with respect to the effects on the monocyte population or the kinetics of reconstitution of the murine myeloid populations or proportion of human immune cell subsets. Given that the optimal regimen was to be selected based on changes in the both the neutrophil and monocyte population (1.1.6) these data should be included in a revised manuscript.

The authors apologize for not reporting the number of animals in our previous manuscript. 3 mice for the vehicle group and 6 mice for CP and/or DS treated groups were used in this study (Fig. 1B). We have included this information in the figure legend of Fig. 1B. In addition, we have re-analyzed our flow cytometry data, and the dynamic changes of murine myeloid populations (i.e. neutrophils and monocytes) upon CP and/or DS treatment, is provided in the revised manuscipt.

3. The protocol for human PBMC transplantation and tumor engraftment lacks sufficient detail to be easily adopted by other labs. Authors should review the critical steps of the protocol to ensure necessary reagents and workflow is well documented. A visual flow figure would be help to convey the model and transplant process.

We thank Reviewer 4 for pointing this issue out. We have included more details regarding PBMC transplantation and tumor engraftment in our protocol section. The authors would also like to note that there will be a video production accompanied by this article that will highlight all essential and critical procedures.

4. Essentially no details or discussion are provided on the methods related to IHC scoring (eg, average CD8, PD1 and PDL1?) or the significance beyond a qualitative assessment of donor suitability. Details of suitable donors are similarly absent, ie for the protocol to be reproducible it would be helpful for other groups to have a better understanding of the demographics of the donor population that were identified as suitable as well as details on immune cell proportions (CD8 and NK at a minimum) and HLA typing. From the current manuscript it is unclear what the authors considered a suitable donor based on the data provided. In addition, were donors suitable across multiple cancer types or was there variability here as well. The data presented in figure 3A and 3B do not appear to include the same donor populations given the variability in the scores although it is unclear which points represent the A431 cells in panel A.

We thank Reviewer 4 for pointing this issue out. We have included more information related to IHC scoring in our revised manuscript. Please also refer to the information below:

Immunohistochemical scoring was performed in a blinded fashion by an experienced pathologist (M. Guo). Immuno-stained sections were initially assessed at low magnification (4x, 10x) to select the core with the highest density of positive cells. TIL markers (indicating immune cell subsets) and human PD-L1 (expressed by tumor cells) were assessed. The area of the entire core occupied by tumor epithelium versus stroma was assessed followed by estimation of the proportion of positive TILs that were intraepithelial or intrastromal (intraepithelial localization defined as lymphocytes within tumor cell nests and/or adjacent to and in direct contact with tumor cells).

Human CD8 and PD-1 expression on tumor-infiltrating leucocytes (TIL) were assessed by assigning an expression score on a 5-point scale (IHC score, range 0–4) within the selected core area at high magnification (20x, 40x). 0, absent; 1, weak intensity/ less than 20% cells; 2, weak-to-moderate intensity/ 20%–50% cells; 3, moderate-to-strong intensity/ 50%–80% cells; 4, strong intensity/ more than 80% cells. Human PD-L1 staining within tumor cells was scored on a 5-point scale (IHC score, range 0–4). 0, absent; 1, weak intensity/ less than 10% cells; 2, weak-to-moderate intensity/10%–30% cells; 3, moderate/ 30%–50% cells; 4, strong intensity/ more than 50% cells.

The authors do not have demographics data of the donor population although the majority of which are of Chinese origin. The authors also do not have details on immune cell proportions of the donated blood and have only performed limited characterization on HLA typing (data not shown). The authors would like to note that a particular PBMC donor might be suitable for one cancer cell line/ PDX but not ideal for others. PBMC donors might therefore need to be screened for each individual cancer model to ensure optimal results.

Each dot in Fig. 3A represents the mean value of 3 mice engrafted with PBMCs from 1 donor, in combination with A431 cells. The authors have screened over 50 healthy PBMC donors in the study, and the results shown in Fig. 3A are representative data from 35 of them. Results in Fig. 3B are representative results of our three established tumor models (i.e. A431, SK-MES-1 and SKOV3), in combination of PBMCs collected from selected donors (protocol 2.4). The authors

also discussed the considerations and criteria of screening suitable donors for a particular model in the revised manuscript.

5. Figure 4 - again it is unclear from the present manuscript how many different donors were used to generate each summary figure for the individual cancer cell lines and PDX lines. It is also concerning that the authors have not used a viability marker to exclude dead cells from the flow cytometric analysis of the TIL population, representative flow plots should be provided which outline the gating strategy used and the typical results.

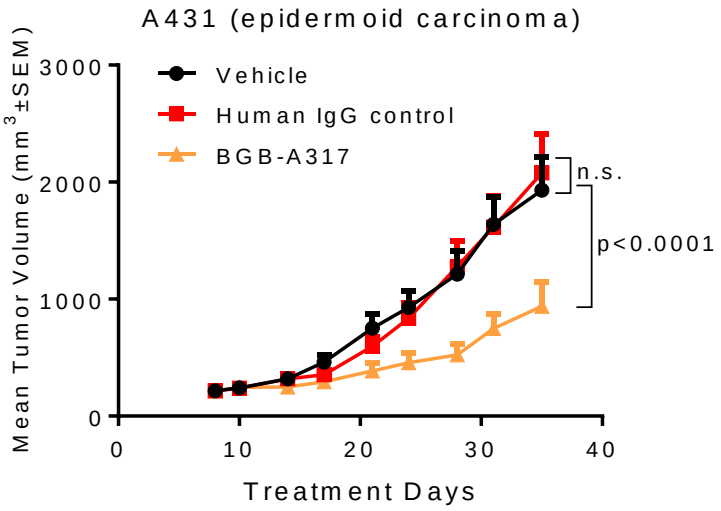
The authors apologize for this confusion. PBMC from one selected donor is used for all the mice in each individual efficacy experiment, i.e. one donor was used to generate each summary figure for the individual cancer cell lines and PDX lines in Figure 4.

The authors acknowledge that, unfortunately, we did not include a viability marker to exclude dead cells for the flow cytometry analysis of TILs. A representative gating strategy we used is shown in Suppl. Fig. 6. We gated our R1 as live cells based on forward and side scatters, and subsequently obtaining the data for CD8+ and CD8+PD+ populations.

6. It is unclear what an isotype control Ab was not used for the I-O agent, could these results reflect a non-specific effect related to the inclusion of a humanized Ab.

The authors thank Reviewer 4 for pointing this out. The authors did use a human IgG control in the beginning for our proof-of-concept studies. An example of the resulting data is provided here for the reviewer's reference (Suppl. Fig. 5). Our data indicate that the anti-tumor effect of BGB-A317 is not due to a non-specific effect related to the inclusion of a humanized Ab. We have latter switched to a vehicle control (i.e. PBS only) for our efficacy studies for the simplicity of experiments. The authors also note that PBS vehicle control has been used by previous literatures for evaluating I-O agent[5,6], and we believe that this would not change the conclusion and interpretation of our results.

Suppl. Fig. 5



Minor Criticisms:

1. The gating strategy for monocytic MDSCs and neutrophils are explained in detail under the protocol and results section. However, the MDSC population, which plays a critical suppressive role in the cancer TME, is not detailed in the background/introduction. Although not essential for

publication, I would recommend explaining the importance of MDSCs in the introduction since they include these populations in the gating strategy.

We thank the reviewer for pointing this out. The authors later realized that the gating strategy used in our Fig. 1 might not be appropriate, and thus revised the related analysis and description in our results and protocol sections. In the revised manuscript, the authors do not show and discuss results related to the MDSC populations.

2. In the discussion (line 268) authors mention that only 20% of tumors/PDx demonstrate acceptable tumor growth rate. Which tumors were acceptable? If only 20%, this model could be of benefit to study which tumors exactly? Is there a correlation between model success and tumor characteristics (i.e. high neoantigen burden, neovascularization, avoiding immune responses, etc.)?

The authors thank the reviewer for pointing this out and we apologize for this confusion. What the authors intended to say is “about 20% of cancer cell lines and PDXs examined demonstrate acceptable tumor growth rate while at the same time having relatively high TILs and PD-L1 staining”. In fact, our humanized mice support the growth of most human tumors tested (as mentioned in the abstract), but a considerable proportion of these have little or no immune cell infiltrations and/or PD-L1 staining, and thus were not further tested in our efficacy studies of I-O therapies. The three human cancer cell lines mentioned in our manuscript, i.e. A431 (epidemoid carcinoma), SKOV3 (ovarian cancer) and SK-MES-1 (lung cancer), as well as two PDX models, i.e. BCLU-054 (lung cancer) and BCCO-028 (colon cancer), are considered good tumor models for I-O therapy evaluation in our humanized mice. The “20%” mentioned in our manuscript was specifically referring to the application of this model for the I-O therapies the authors have explored in this study. We believe that with further understanding and characterization of this model, just as the reviewer pointed out, the scientific community will expand its application to additional therapeutics and tumor types.

The authors also thank the reviewer’s question regarding the correlation between model success and tumor characteristics. We did not explore this but it is an interesting question to be investigated in future studies.

3. HLA matching (line 271): it seems like HLA matching would be a key step for other studies that using this xenograft model. HLA…"Might not be the determining factor" is a vague response. They should at least include the data and explain why HLA matching might not be critical step.

The authors acknowledge that this is only a preliminary observation and we have not performed detailed analysis to fully support this claim. Therefore, we have removed the related claims in our revised manuscript.

4. Authors should cite original work instead of review articles.

We thank the reviewer for pointing this out and we have revised our manuscript to include more original research articles.

5. Page 1 ln 1 - "human PBMC-based humanized" seems redundant

We thank the reviewer for pointing this out and we have removed “humanized” in this phrase.

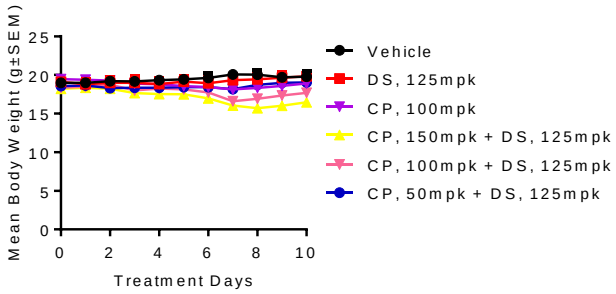
6. Rationale or reference for use of disulfiram in myeoablation strategy should be provided.

We thank the reviewer for pointing this out and we have explanined this in the protocol 1.1.3 of our revised manuscript. Disulfiram (DS) blocks the urotoxicity of cyclophosphamide (CP) in mice, and CP combined with DS was suggested to have longer-lasting neutropenia than

animals treated with CP alone[4]. We also observed that there is decreased number of death events from mice treated with CP+DS versus CP alone (Suppl. Fig. 3).

Suppl. Fig. 3

Group	Mortality
Vehicle	0 (of 3)
DS, 125mpk	0 (of 6)
CP, 100mpk	5 (of 6)
CP, 150mpk + DS, 125mpk	5 (of 6)
CP, 100mpk + DS, 125mpk	0 (of 6)
CP, 50mpk + DS, 125mpk	1 (of 6)



7. Clone numbers should be provided for all Antibodies used.

We thank the reviewer for pointing this out and we have revised our manuscript accordingly.

8. Genetic background or source of NOD/SCID animals should be listed

We thank the reviewer for pointing this out and we have added this information to our protocol section 1.1.1.

9. No scale bar provided in images. This would be more helpful than magnification.

We thank the reviewer for pointing this out and we have included the scale bar in our revised manuscript (Fig. 2C)

10. Fig 2 is confusing. Are these animals CP treated alone or did they receive CP+DS, legend of panel B suggests CP only. Furthermore, panel A suggests then animals receive treatment after implantation yet the data in this figure does not appear to be in a model that receives any additional treatment.

The authors apologize for this confusion. We thank the reviewer for pointing this out and we have revised the legends of Fig. 2. The “treatment” in Fig. 2A refers to I-O therapy treatment while “treatment” in Fig. 2B was intended to mean “CP+DS treatment”. We have corrected the misleading legends. More specifically, the red line in Fig. 2B represents data from the group with PBMC and tumor transplation but without CP or DS treatment (supposedly poor humanization), while the blue line represent data from mice with PBMC+tumor+CP+DS.

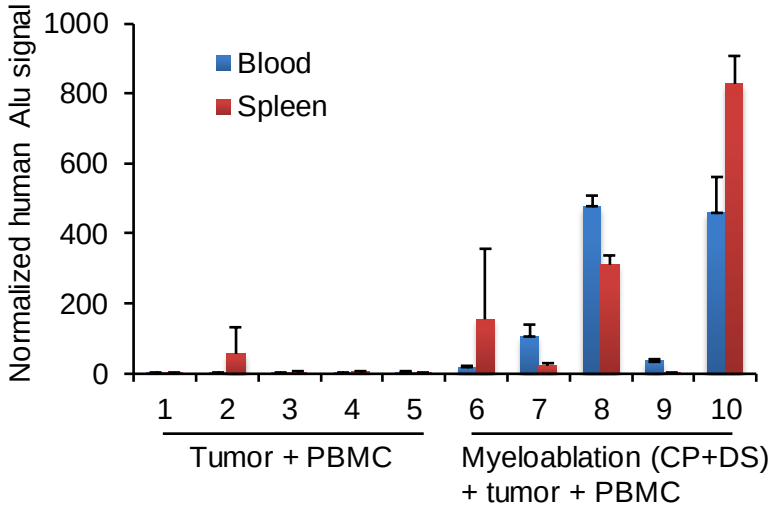
It is not clear when the tumor sections were collected for IHC.

In PBMC donor and cancer cell line/PDX screens, tumors were harvested at an average volume of 200-500 mm^3, and then processed for IHC stainings. We have added this description in protocol section 2.3 and 3.1 of our revised manuscript.

It is not clear whether the myeoablation strategy results in increased number of CD8+ T cells that are able to engraft in these mice or a preferential recruitment/expansion/survival of these cells within the implanted tumor.

We thank the reviewer for pointing this out. We have IHC data demonstrating a preferential recruitment/expansion/survival of human immune cells within the implanted tumors with prior treatment of CP+DS (Fig. 2C). In addition, CP+DS pre-treated, PBMC-engrafted mice demonstrate significant elevation of human Alu sequence signal in the blood and spleen compared to the non-CP treated mice, quantified by RT-PCR at 14 days post subcutaneous PBMC injection (Suppl. Fig. 1). This suggests potentially more systemic presence of human immune cells in the CP+DS-treated mice.

Suppl. Fig. 1



11. The authors state: The dose regimen of cyclophosphamide might need to be pre-determined prior to actual studies and was found to vary slightly between different immunodeficient mouse strains". This seems to be an important finding but data in support of this statement is not provided.

The authors would like to note that this is only an observation and have not been characterized in details. We have some preliminary data suggesting that the more immuno-compromised strains, e.g. NCG mice, might require lower doses of CP. However this is not a conclusion and therefore, we do not plan to elaborate on this in our discussion. We have revised our statement in the manuscript accordingly.

12. Page 6 ln 231 "NOD/DCID"

We thank the reviewer for pointing this out and we have corrected this typo.

13. Statistical tests were not reported or discussed

We thank the reviewer for pointing this out and we have included description of statistical test in the figure legends.

1 Zhang, T. *et al.* The binding of an anti-PD-1 antibody to FcgammaRIota has a profound impact on its biological functions. *Cancer Immunol Immunother.* **67** (7), 1079-1090, (2018).
2 Dunay, I. R., Fuchs, A. & Sibley, L. D. Inflammatory monocytes but not neutrophils are necessary to control infection with Toxoplasma gondii in mice. *Infect Immun.* **78** (4), 1564-1570, (2010).
3 Ghasemlou, N., Chiu, I. M., Julien, J. P. & Woolf, C. J. CD11b+Ly6G- myeloid cells mediate mechanical inflammatory pain hypersensitivity. *Proc Natl Acad Sci U S A.* **112** (49), E6808-6817, (2015).
4 Gamelli, R. L., Ershler, W. B., Hacker, M. P. & Foster, R. S. The effect of disulfiram on cyclophosphamide-mediated myeloid toxicity. *Cancer Chemother Pharmacol.* **16** (2), 153-155, (1986).
5 Fisher, T. S. *et al.* Targeting of 4-1BB by monoclonal antibody PF-05082566 enhances T-cell function and promotes anti-tumor activity. *Cancer Immunol Immunother.* **61** (10), 1721-1733, (2012).
6 McCormack, E. *et al.* Bi-specific TCR-anti CD3 redirected T-cell targeting of NY-ESO-1- and LAGE-1-positive tumors. *Cancer Immunol Immunother.* **62** (4), 773-785, (2013).
7 Sasaki, E. *et al.* Development of a preclinical humanized mouse model to evaluate acute toxicity of an influenza vaccine. *Oncotarget.* **9** (40), 25751-25763, (2018).
8 Tobin, L. M., Healy, M. E., English, K. & Mahon, B. P. Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. *Clin Exp Immunol.* **172** (2), 333-348, (2013).
9 Pearson, A. T. *et al.* Patient-derived xenograft (PDX) tumors increase growth rate with time. *Oncotarget.* **7** (7), 7993-8005, (2016).
10 Pu, X. *et al.* Patient-derived tumor immune microenvironments in patient-derived xenografts of lung cancer. *J Transl Med.* **16** (1), 328, (2018).

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Line 41-48: Rephrased.

Line 176-178: Section 4.2 of the protocol represents a standard procedure for immunohistochemistry and the authors do not think necessary to re-write this step. The authors have nevertheless rephrased some of the statements.

Line: 244-247: Section 5.6 of the protocol represents a standard procedure for obtaining single cell suspensions for FACS analysis and the authors do not think necessary to re-write this step. The authors have nevertheless rephrased some of the statements.

Line 353-356: Rephrased.

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VOLUNTARY BLOOD DONATION FOR RESEARCH USE
自愿献血供研究使用

BeiGene (Beijing) Co., Ltd.
百济神州（北京）生物科技有限公司

Study Title: TESTING AND CHARECTERIZATION OF IMMUNE-REGULATORY AGENTS IN HUMAN IMMUNE CELLS AND CANCER IMMUNOLOGY RELATED ISSUES
实验题目：免疫调节药物分子对人体免疫细胞的作用机理和调控效应的研究及有关肿瘤免疫学问题的探索。

You are being asked to provide some blood for a research study. Your participation is completely voluntary.
本实验希望您能献血供研究使用。您的参与完全基于自愿。

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无论您是否同意献血，是否决定将血液供研究使用，都不会损害您的利益。

- ***What is the purpose of this research?***
  本研究的目的是什么？

The purpose of this study is to investigate how human immune cells respond to immune--modulating agents in vitro, and how it affects on cancer cells, as well as other cancer immunology-related questions.
本实验的目的在于研究人体免疫细胞在体外实验中对免疫调节药物分子有何反应，对癌症细胞有何影响，以及其他有关肿瘤免疫的问题。

- ***What does participation in this research involve?***
  参与本实验需要做什么？

Your participation in this research project is to provide blood samples during the period from _____ to _____ as scheduled below.
本实验需要您于____年____月____日至____年____月____日期间按照以下时间表提供血液样本。

Date 日期	Amount of the blood to be collected (cc or mls) 采血量（毫升）

The blood collection will strictly follow the schedule above. If you are required to provide blood at any other time, you may choose to (i) refuse such requests, or (ii) sign a revised consent form.
血液收集将严格按照上述时间表进行，如您被要求在时间表以外的时间进行献血，您可以选择(i)拒绝该等要求，或(ii)重新签署变更后的知情同意书。

The blood collected will be used for research only. It **will not** be used for:
本次收集的血液仅供研究使用，不会用于：

1. Regular health check items such as white blood cell counts, lipid, vitamin and cholesterol level, etc.
   常规体检项目，如白细胞计数、血脂、维生素和胆固醇水平等
2. Viral carrier, anti-viral antibody titer, etc.
   病毒携带情况、抗病毒抗体浓度测定等
3. DNA or cDNA sequencing to determine any genetic information.
   DNA 或 cDNA 测序以确定个人的遗传和基因信息
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   人事部门备案的雇佣和绩效考评管理信息

In addition, the research may involve the use of certain health information, such as recent flu or other acute infectious diseases. This is to ensure donor is in good health condition. Please fill out the attached Blood donor's health info sheet truly and completely.
此外，为了保证献血者身体健康，本研究可能会使用您的健康信息，如近期患流感或其他急性传染病的情况。请您如实完整地填写随附献血者健康信息表。

- ***How the blood sample will be stored, used or destroyed?***
  血液样本将如何保存、使用或销毁？

Your blood sample will be kept in 4 degree C refrigerator, and used for cancer immunology and immune-regulatory agent testing and research until study activities are completed. If some cases, if there are PBMCs or T-cells isolated from the blood samples not used up, the unused blood will be stored at -80 degree C for later time to use in the same type of study and research. Any of the blood sample-derived immune cells will be regarded as expired after storage for one year, and discarded as bio-waste.
您的血液样本将保存在 4℃的冰箱中，用于癌症免疫和免疫调节药物分子的检测和研究，直到研究活动完成。如果血液样本中分离出来的外周血单核细胞或 T 细胞有剩余，未使用的剩余部分将 保存在-80℃环境中，供以后的同类试验和研究使用。任何血液样本中提取的免疫细胞在保存一年后将视为过期，会作为生物废弃物处理。

- ***Are there any benefits participating in this research?***
  参与这项实验有哪些福利?

There are no benefits to you should you decide to participate in this study. You will not receive any results from tests done on your blood, because they have no reliable meaning at this time.
参与本实验没有任何福利。您不会收到任何血液检测结果，因为这些没有实际参考意义。

- ***Are there any risks from participating in this research?***
  参与这项实验对身体有不良反应吗？

A risk of providing blood is mild to moderate pain at the site of the needle puncture into your vein. Other risks are redness, minor bleeding, swelling and a bruise at the site of the needle puncture or, rarely, an infection. Some people feel dizzy or faint when blood is taken; however, most people do not experience any problems.
献血的一个不良反应是，针刺进血管的部位会产生轻微至中度疼痛。其他反应有针刺部位红、肿、少量出血、淤青，极少情况下会出现感染。抽血时，有人会感到头晕，甚至昏厥，但是大部分人不会有任何问题。

- ***Other important information you should know.***
  您应该知晓的其他重要信息。

✓ Any identity information or health information you may provide will be kept confidential, unless its disclosure is permitted by law. Information created or collected in this research may be shared with other researchers, but none of it can be traced back to you. Your name will not be used in any publication or presentation that may result from this research.
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- ✓ You will not receive any compensation if the results of this research are used towards the development of a commercially available product.
  您不会收到任何补贴，即使本实验的结果用于开发有上市可能性的产品。

- ✓ You will not be paid to participate in this study because the donation is completely on your willingness and voluntary base. However, BeiGene will make a small amount of nutritional compensation the donor.
  参加本研究是无偿的，因为献血是您的自愿行为。但是，百济神州将给献血者发放少量营养费。

- ✓ You may choose to withdraw your consent to provide blood at any time. You may withdraw your blood from use in this research at any time. If you wish to withdraw your consent or to remove your blood from use in the study, please contact the researcher in writing. If your blood has not already been used up by the researchers, any blood that remains will be destroyed.
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  Telephone number of Kang Li: +86 10 5895 8231, Lai Wang, 86 10 5895 8221
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Consent: I have read the above information and have been given an opportunity to ask questions. I agree to provide blood for this study **"TESTING AND CHARECTERIZATION OF IMMUNE-REGULATORY AGENTS IN HUMAN IMMUNE CELLS AND CANCER IMMUNOLOGY RELATED ISSUES".** I consent voluntarily to have my blood samples stored and used in the manner and for the purpose indicated above, and I have been given a copy of this signed consent form for my own records.
同意：我已阅读以上信息，并有权提出问题。我同意为本研究**"免疫调节药物分子对人体免疫细胞的作用机理和调控效应的研究及有关肿瘤免疫学问题的探索"**献血。我自愿同意按照以上所述目的及方式储存和使用本人血液样本。我已收到一份已签名的同意书供我参考。

Participant's Printed name
参与人姓名

Signature
签名

Date
日期

Blood donor's health info sheet
献血者健康信息表

Your Name / 姓名:	
Gender / 性别:	
Department / 部门:	

Please check on the answer to following questions 请勾选以下问题的答案	Yes 是	No 否
1. Are you having flu, diarrhea and other infectious diseases? 目前您是否患有流感、腹泻或其他传染病？		
If yes, please stop here. You may come back after recovered. 如果是，请停止作答。您可以在恢复健康后再参加本研究。		
2. Have you experienced hepatitis B, or hepatitis A viral infection in last one year? 在过去一年中您是否患过乙肝或甲肝?		
3. Have you received any treatment for HBV, HCV and HIV infections, or receive any medications for the infections in last one year? 您是否接受过任何针对HBV、HCV 或HIV 感染的治疗，或最近一年中是否接受过任何针对上述感染的药物治疗？		