ARRIVE

<table>
<tr><td>Title</td><td colspan="3">Inhibitory effect of rBCG containing the fusion gene BFNA on EBV-positive tumours</td></tr>
<tr><td rowspan="3">Study design</td><td>Group</td><td>Experimental Group</td><td>Control Group</td></tr>
<tr><td>Group Setting</td><td>Epstein-Barr virus positive tumor-bearing mice injected with BCG, BCG-EBNA1, BCG-BZLF1, pMV-BFNA-rBCG</td><td>Epstein-Barr virus positive tumor-bearing mice injected with PBS</td></tr>
<tr><td>Experiment Unit</td><td colspan="2">a cage of 6 mice</td></tr>
<tr><td rowspan="2">Sample size</td><td>30 6-week-old female mice in total</td><td>24 6-week-old female mice randomly divided into 4 groups, with 6 mice in each group</td><td>6 6-week-old female mice in one group</td></tr>
<tr><td>How to Decide</td><td colspan="2">While protecting animals and complied ethical needs, strive to obtain reproducible and valuable data as much as possible.</td></tr>
<tr><td>Inclusion and Exclusion Criteria</td><td colspan="3">a. healthy female mice; b. aged 6 weeks; c. weigh 19-20g; d. 9 days of tumor vaccination; e. tumor size was basically the same.</td></tr>
<tr><td rowspan="3">Randomization</td><td colspan="3">a. The physiological states of all mice were similar, and the cages were randomly placed before animal numbering. The placement of the cages and numbering of mice were different.
b. The experimental group and the control group were randomized into groups according to a completely randomized design. We numbered 30 mice one by one and used a random number table to group them. Randomly group are as follows：</td></tr>
<tr><td rowspan="2">Randomized Group</td><td>Experimental Group</td><td>Control Group</td></tr>
<tr><td>Group A：5 6 11 19 21 27
Group B：1 4 10 13 28 29
Group C：7 8 15 20 25 26
Group D：3 9 12 16 18 23</td><td>2 14 17 22 24 30</td></tr>
<tr><td>Blinding or Masking</td><td colspan="3">Shuyang Shao placed the cages; Shuo Huang numbered the mice; Shuyang Shao, Shuo Huang, Junying Wang, Liding Fan and Furen Meng performed the experiment; Yuanhui Wang integrated the data; Qingjie Xue and Li Zhang evaluated the outcome.</td></tr>
<tr><td>Outcome Measures</td><td colspan="3">The effect of recombinant BCG on the survival time of the tumor-bearing mice was observed, and the necrosis and apoptosis of the tumor cells were detected by Flow cytometry staining. The specific CTL killing effect of rBCG was detected by CCK-8 method, and the inhibitory and preventive effects of rBCG on transplanted tumor in nude mice were observed In vivo optical imaging system analysis detection: non-radioactive labeled fusion protein was used to observe and quantify the anti-tumor effect and various biological processes in vivo. The survival time, tumor formation time, tumor volume and weight of mice were measured and recorded.</td></tr>
<tr><td>Statistical Methods</td><td colspan="3">The tumorigenesis time, tumor volume and weight of the mice are expressed as the mean ± standard deviation (x ±s), and SPSS 19.0 was used to process and analyze the measurement data. The tumorigenesis time data of mice were examined by the log-rank test. The number of independent samples was compared by single-factor analysis of variance (ANOVA), the volume of tumors in mice was compared by repeated-measures ANOVA, and the cytotoxicity to spleen cells in mice was compared by ANOVA using a factorial design. P≤0.05 was considered to indicate statistical significance.</td></tr>
<tr><td>Experimental Animals</td><td colspan="3">Source of animal: SPF
Species of strain: C57BL/6
Specification: 6 weeks
Number: 30 female mice
Weight: 19-20g</td></tr>
<tr><td>Experimental</td><td>Entering Date：May</td><td>Application Date：Jan. 01, 2020</td><td>Ending Date：Dec. 01, 2024</td></tr>
</table>

Procedures	06, 2020		
	We obtained BZLF1 and EBNA1 cDNA, and overlapped jointly to assemble the fusion gene BFNA. Then pMV-BFNA was transformed into BCG-competent cells after inserting BFNA into pMV38. With western blotting to detect the target fusion protein, specific antibodies were detected in serum by ELISAs and spleen cell-specific cytokines were detected by ELISPOT. The experimental group was injected with equivalent dose of BCG, BCG-EBNA1, BCG-BZLF1, pMV-BFNA-rBCG； and the control group was injected with equivalent dose of PBS. CTL activity, tumor weight, tumor formation time and mouse survival were analyzed in EBV-positive tumor cell (NPRC18) cancer models, and flow cytometry was performed to analyze the quantities of $CD8^+$ and $CD4^+$ T cells in C57BL/6J mice. Single-factor analysis of variance was performed with SPSS 19.0 to evaluate rBCG inhibition. Execution method: euthanasia (We followed the method of rodent execution that was more common in the international community: the experimental animals were simultaneously placed in a sealed box container, then the box was slowly filled with CO2, and the animals died within 1 to 3 min in the CO2-filled container.)		
Results	The molecular weight of the fusion protein was approximately 55.5 kD. The titer of antibody in the rBCG group was highly significant ($P \leq 0.01$) and prolonged that tumorigenesis time, the specific killing ability targeting the recombinant target protein was increased. The rBCG group with the BFNA fusion gene demonstrated a better effect on tumors than BCG-EBNA1 and BCG-BZLF1 groups. Based on flow cytometry analysis, the numbers of $CD4^+$ T and $CD8^+$ T cells in the blood of the rBCG group were significantly higher than the control group ($P < 0.01$). The mice injected with rBCG had more obvious lymphocyte infiltration in the tumor area.		