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JL-Lightning CAR-T，a Next Generation Non-viral CAR-T without In Vitro Culturing Procedure, Shows High Clinical Efficacy, Good Safety with Low Dosage

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SUMMARY

Background: CAR-T with high *in vivo* proliferation and long persistency could benefit hematologic malignancy patients with low relapse and long survival. Previous studies reveal that CAR-T stemness (T-stem) and exhaustion levels are major determinants of CAR-T quality that contribute to its expansion ability and duration.. However, conventional manufacturing process with 9-14 days of *in vitro* culture sacrifices T-stem and prolong the vein-to-vein process. Researchers have tried to shorten this process, but many failed due to T-cell activation insufficiency, transfection toxicity, and product safety concerns, especially in non-viral transfection products.

Methods: Two versions of non-viral JL-Lightning CAR-T without in vitro culture process have been developed，with the fast manufacturing process of 30 h and 6 h, respectively. JL-genome writing technology has been developed and proven with high transfection efficiency and low toxicity on both versions of JL-Lightning CAR-T (V-30h & V-6h). We generated a tri-targeting CAR molecules (BZE2204) using VHHs against CD19, CD22 and BCMA, then evaluated its safety and efficacy *in vitro* and *in vivo* using Raji orthotropic model and MM1S orthotropic model. One investigator-initiated trial (IIT) is designed to assess the safety and preliminary efficacy of BZE2204 CAR-T in relapsed/refractory Non-Hopkins Lymphoma (r/r NHL) patients.

Results and Conclusion: *In vitro* experiments showed both versions of BZE2204 exerted strong cytotoxicity on CD19-, CD22- and BCMA-positive tumor cells with high CAR-T cell expansion rates, superior stemness, and low exhaustion phenotype compared with conventional CAR-T. V-6h, which has the shortest process time, displayed the highest T-stem and proliferation among all groups. In vivo models showed V-30h BZE2204 inhibited tumor growth and significantly prolonged mice lifespan under super low dosage (1E5 CAR-T/mouse). Six r/r NHL patients with high tumor burden were enrolled and infused with BZE2204 CAR-T (three for V-30h and three for V-6h) with the dosage of 40-fold lower than conventional CAR-T. The data showed that low dosage CAR-T BZE2204 had promising clinical response with manageable safety profile, with overall response rate (ORR) of 100% and complete remission (CR) of 67%, four achieved CR, and one with a single tumor over 7000mm2 in-size achieved partial remission (PR) with >80% of tumor shrinkage. Pharmacokinetics (PK) data showed BZE2204 CAR-T proliferation is much higher than other CAR-T products on market.

Our results show JL-Lightning CAR-T achieves high anti-tumor efficacy and good safety in large tumor-burden r/r NHL patients with super low dosage, likely due to the strong proliferation capability of JL-Lightning CAR-T caused by no post-transfection *in vitro* culture, as well as the unique design of multi-VHH CAR . It offers a more effective, affordable and short vein-to-vein process option for CAR-T treatment of hematologic malignancies.

PRODUCT DESIGN & TECHNOLOGY PLATFORM

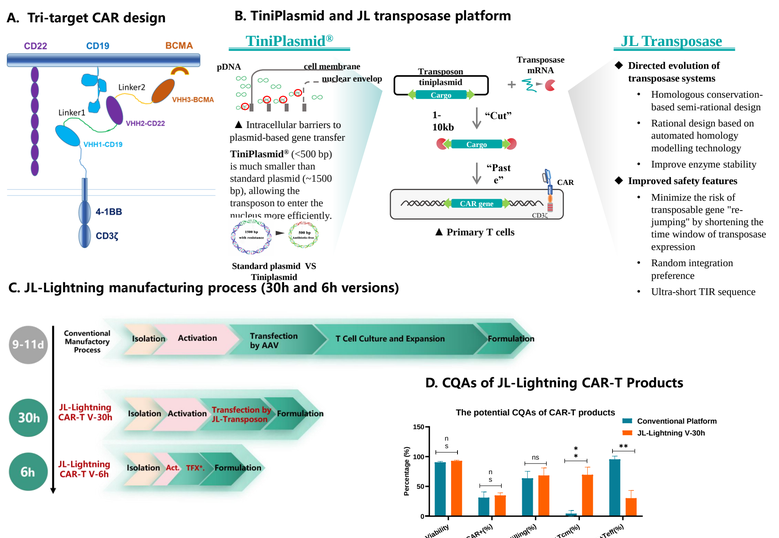

Fig.1 Product design & Tech Platform.
A) Schematic Diagram of the Tri-Targeted CAR-T Structure. Anti-CD19, CD22 and BCMA VHH are formed in series, 4-1BB act as costimulatory domain; **B)** Delivery System with Transposase mRNA and Tiniplasmids. **C) Scheme of JL-Lightning manufacturing Process**. Our super-fast manufacturing system shorten the activation and transfection process and have no post-transfection cell culture before formulation. Short culture process normally gets higher stem-like phenotype of CAR-T cells and also lead to better proliferation **(D).**

PRE-CLINICAL RESULTS

Part 1. Product CQAs with JL-Lightning CAR-T Manufacturing Platform

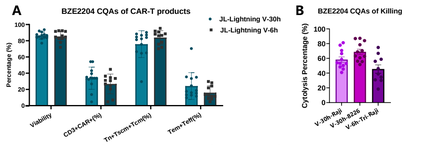

Fig.2 CQAs of BZE2204 CAR-T DP (Patient sample data with V-30h version and V-6h version). A) Both versions of BZE2204 products have high cell viability, good editing efficiency and high percentage of T cell with memory phenotype (Tscm and Tcm); **B)** BZE2204 CAR-T products show high and consistent antigen-specific tumor cell killing.

Part 2. Product CQAs with JL-Lightning CAR-T Manufacturing Platform

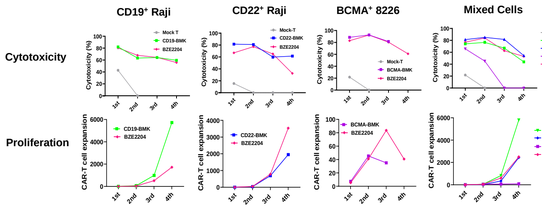

Fig.3 In Vitro Tumor Cell Killing and CAR-T Proliferation Analysis. A) BZE2204 tri-targeted CAR-T shows comparable tumor cytotoxicity with CD19- ,CD22- and BCMA- CAR-T benchmark(BMK).

BZE2204 tri-targeted CAR-T shows higher proliferation potential than CD22 and BCMA- CAR-T BMK under tumor stimulation.

Part 3. Pre-clinical data for Raji (CD19+CD22+) and MM1S (BCMA+) tumor models

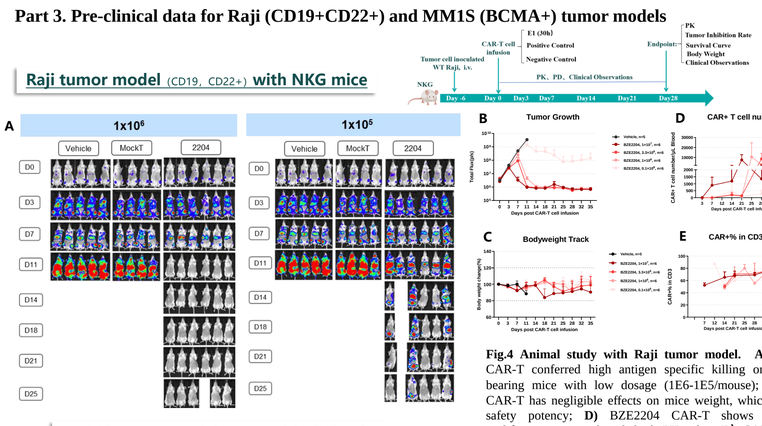

Fig.4 Animal study with Raji tumor model. A-B) BZE2204 CAR-T conferred high antigen specific killing on Raji tumor-bearing mice with low dosage (1E6-1E5/mouse); **C)** BZE2204 CAR-T has negligible effects on mice weight, which shows good safety potency; **D)** BZE2204 CAR-T shows high in-vivo proliferation potential with high PK value; **E)** CAR-T% in CD3 demonstrates the proliferation in panel D is antigen specific.

Fig.5 Animal study with MM1S tumor model. A-B) BZE2204 CAR-T conferred high antigen specific killing on MM1S tumor-bearing mice with low dosage; **C)** BZE2204 CAR-T has negligible effects on mice weight, which shows good safety potency; **D-E)** BZE2204 CAR-T shows high in-vivo proliferation potential for both CD3-T cells (D) and CAR-T cells (E).

Of note, we found that our Tri-targeted BZE2204 CAR-T shows better anti-tumor efficacy than BCMA-CAR-T with the same BCMA-CAR domain. PK data also confirmed this phenomenon with much higher proliferation with BZE2204 than BCMA CAR-T.

CLINICAL RESULTS

Part 1. Clinical Pharmacokinetics Data for BZE2204 (V-30h and V-6h)

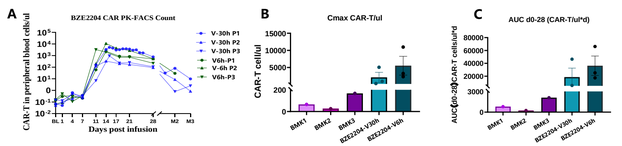

Fig.6 Pharmacokinetics Analysis of BZE2204 A) BZE2204 CAR-T products show high antigen-specific CAR-T proliferation after re-infusion back to patients. Three patients in V-30h group and three patients in V-6h group; **B-C)** BZE2204 CAR-T displayed higher pharmacokinetics(PK) than CAR-T products on-sale (BMK1-3).

Part 2. Clinical Data for BZE2204 (V-30h and V-6h)

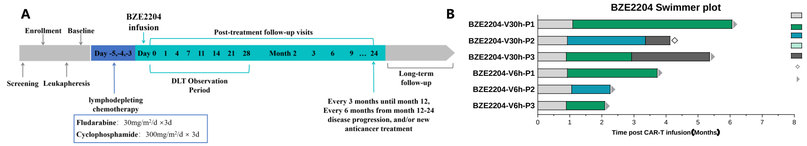

Fig.7 Clinical Output of BZE2204 (V-30h and V6h).
A) Clinical treatment and follow-up schedule for BZE2204 investigator-initiated clinical trials (IIT) ; B) Swimmer plot of BZE2204 CAR-T V-30h and V-6h) patients. C) Best change of tumor volume for BZE2204 V-30h and V-6h patients.

These data show that BZE2204 CAR-T holds promising clinical response with manageable safety profile with low (5E4 CAR-T/kg). Six patients (3 for V-30h and 3 for V-6h) achieved ORR=100% and CR=67% (Four achieved complete remission (CR), and one with a single tumor over 7000mm2 in-size achieved partial remission (PR) with >90% of tumor shrinkage. All patients have G1 CRS, only one has G3 ICANs and got remission 1 day after clinical intervention.

Part 3. Representative Image for BZE2204 IIT Clinical Trial (V-30h and V-6h)

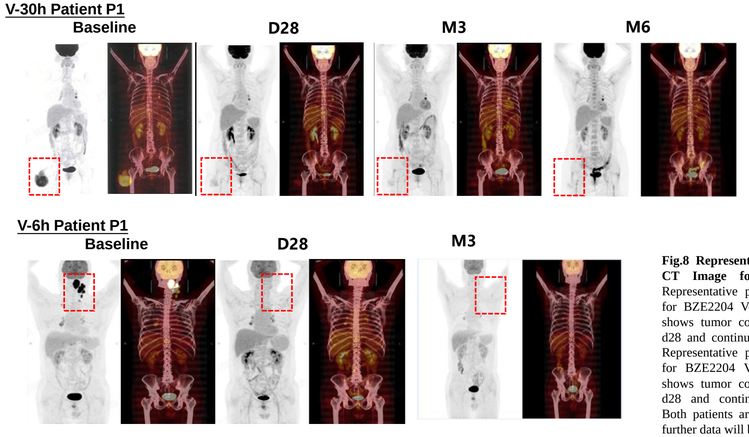

Fig.8 Representative Patient PET-CT Image for BZE2204. A) Representative patient PET-CT data for BZE2204 V-30h platform. Data shows tumor complete response on d28 and continue response (M6) **B)** Representative patient PET-CT data for BZE2204 V-6h platform. Data shows tumor complete response on d28 and continue response (M3). Both patients are still in group and further data will be collected.

CONCLUSION

1. JL-Lighting CAR-T manufacturing platform shows high viability, high editing efficiency and exhibit higher memory phenotype percentage in CAR-T cell product.
2. BZE2204 CAR-T demonstrate high proliferation and potent cytotoxicity in *in vivo* tumor models, with good safety profile.
3. Clinical study with limited data showed BZE2204 have high pharmacokinetics (PK) and high efficacy with 100% overall response rate (ORR) and high complete response rate. The study is ongoing with active patients' enrollment.