

Engineered T Cells Secreting α B7-H3- α CD3 Bispecific Engagers for Enhanced Anti-Tumor Activity Against B7-H3 Positive Multiple Myeloma: A Novel Therapeutic Approach

Punchita Rujirachaivej^{1#}, Teerapong Siriboonpiputtana^{2#}, Kornkan Choomee^{3,4}, Kamonlapat Supimon^{3,4}, Thanich Sangsuwannukul⁵, Pucharee Songprakhon^{3,4}, Krissada Natungnuy^{3,4}, Piriya Luangwattananun^{3,4}, Pornpimon Yuti^{3,4}, Mutita Junking^{3,4*}, Pa-thai Yenchitsomanus^{3,4*}

¹Graduate Program in Clinical Pathology, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

²Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

³Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

⁴Division of Molecular Medicine, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

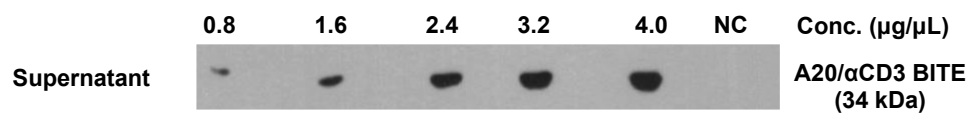
⁵Department of Molecular Medicine, Mayo Clinic Rochester, MN USA

These two authors contributed equally to this work.

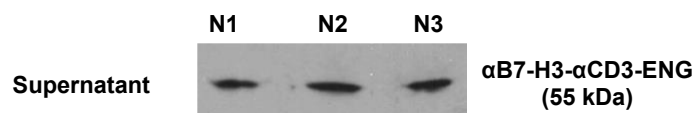
*Corresponding authors: Mutita Junking (E-mail: mjunking@gmail.com; mutita.jun@mahidol.ac.th), Pa-thai Yenchitsomanus (E-mail: ptyench@gmail.com; pathai.yen@mahidol.edu)

Supplementary Information

A

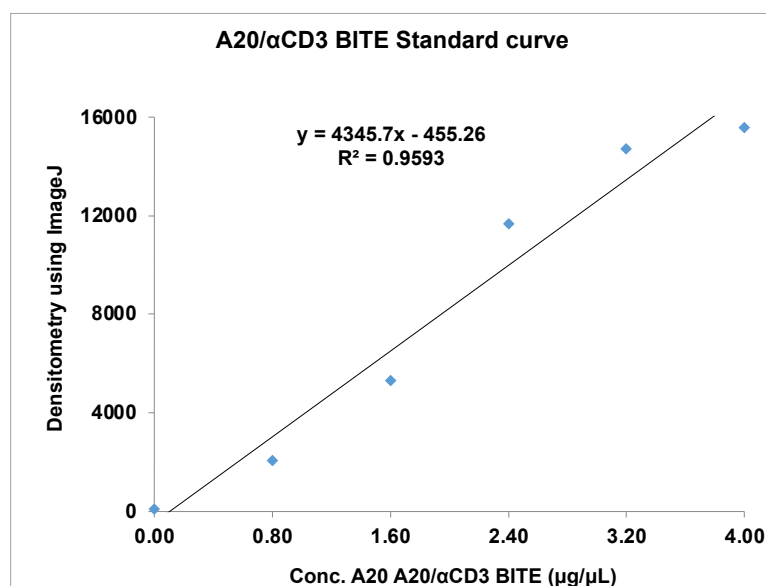


B



C

Conc. A20 A20/αCD3 BITE (μg/μL)	Densitometry using ImageJ
NC	94.9
0.8	2062
1.6	5305.0
2.4	11680.3
3.2	14703.8
4.0	15570.5

D**E**

α B7-H3- α CD3-ENG	Densitometry using ImageJ	Conc. α B7-H3- α CD3-ENG (μg/mL)
N1	7156.69	38.55
N2	9200.93	50.31
N3	8904.83	48.61
Mean	8420.82	45.82

Fig.1 BITE protein concentration in culture supernatants, assessed through semiquantitative immunoblotting analysis and compared to known A20/αCD3 BITE concentrations. Immunoblotting utilizing an anti-c-Myc tag antibody identified **(A)** A20/αCD3 BITE and **(B)** αB7-H3-αCD3 BITE proteins in the culture supernatants of stable cells. **(C)** Densitometry data of A20/αCD3 BITE protein bands relative to GAPDH bands on SDS-PAGE were analyzed using ImageJ software to generate **(D)** the standard curve. Subsequently, **(E)** the concentration of αB7-H3-αCD3 BITE protein in culture supernatants was calculated from the A20/αCD3 BITE standard curve, utilizing the densitometry data of αB7-H3-αCD3-BITE protein bands relative to GAPDH bands on SDS-PAGE, also analyzed via ImageJ software.