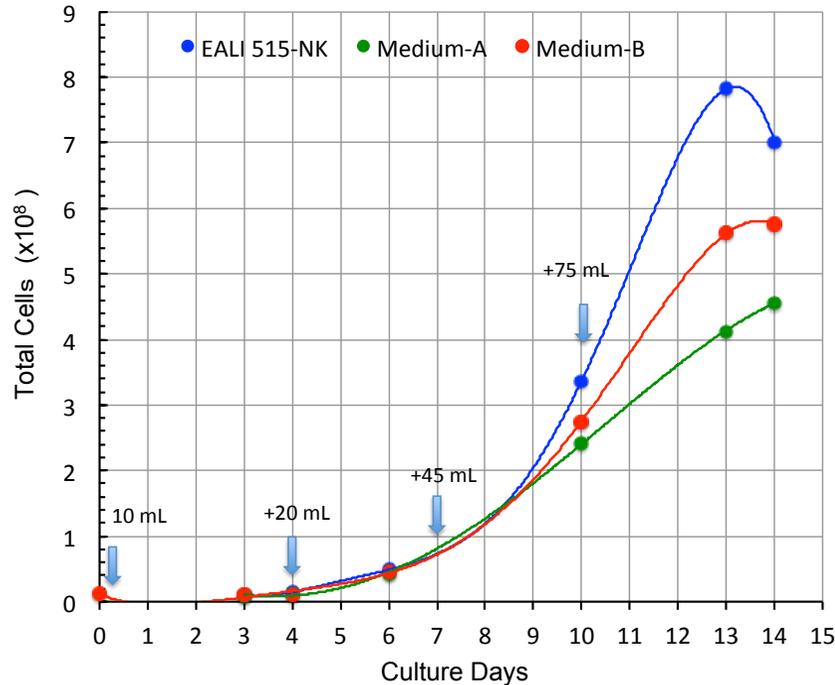


Comparative study of cell growth and killing activity of NK cells cultured with three kinds of media

Experiment

- ① Cell growth stimulation test
- ② Flow Cytometrical Analysis
- ③ Cell killing activity against K562 tumor cell

Comparative study of cell proliferation ability between three types media, EALI 515-NK, Medium-A and -B.



Method: Peripheral Leucocytes (1.18×10^7) were cultured in T75 culture flask pre-treated anti-Her2 antibody with 10ml of each medium that was containing 10% plasma, and cultured in the incubator of 5% CO₂/Air at 37°C for 14 days.

New media were added at the day indicated on the Figure, and maintained cultures until day 14 with the medium of 150 mL/flask.

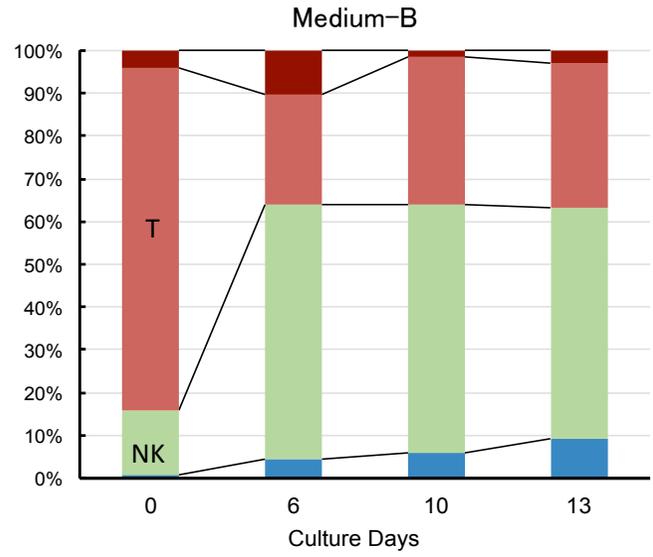
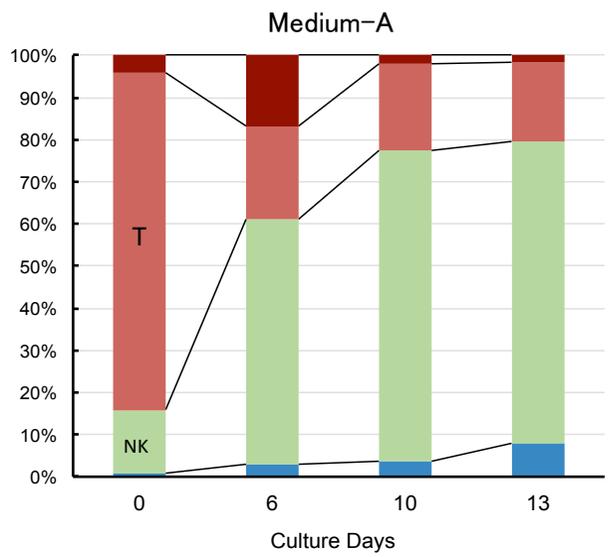
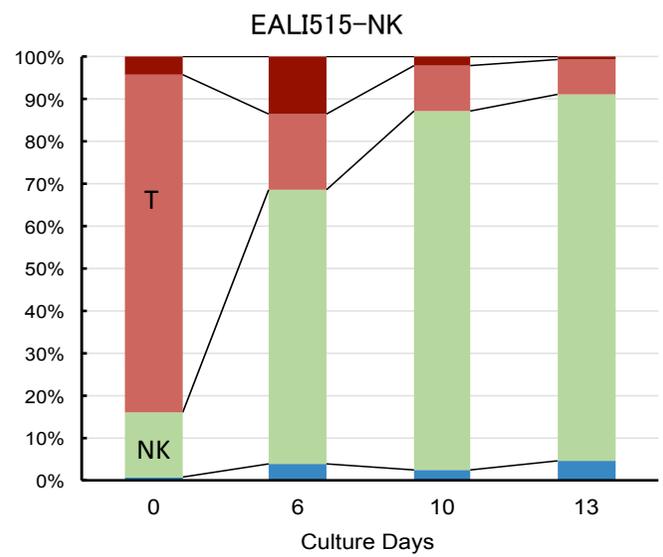
The maximum growth was obtained by the culture with EALI 515-NK medium, and the total cell number was 7.8×10^8 at day 13.

*** EALI 515-NK medium support the higher cell growth than commercial available regular media**

Flow-cytometrical analysis of cell surface antigens that were cultured with three types

	% positive Cells at day 13		
	EALI515-NK	Medium-A	Medium-B
CD56+ cells	91.2%	79.7%	63.1%
CD56-,CD3+ cells	8.2%	18.6%	33.9%

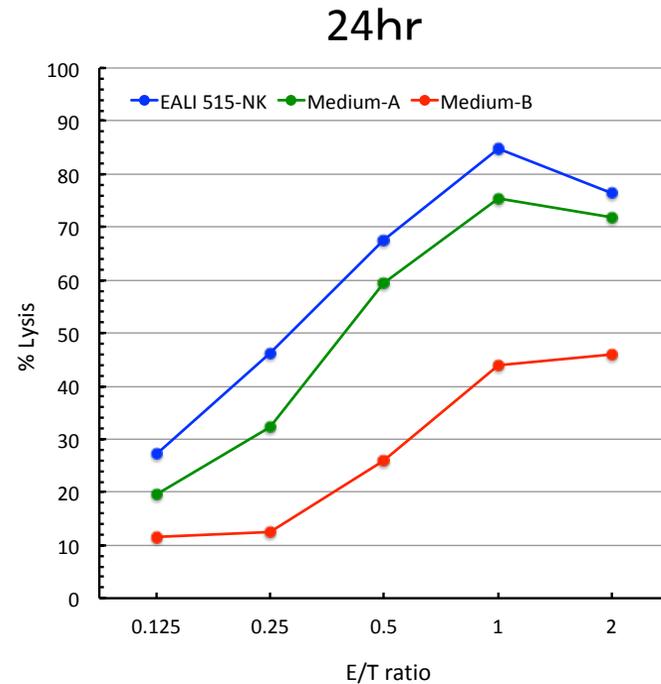
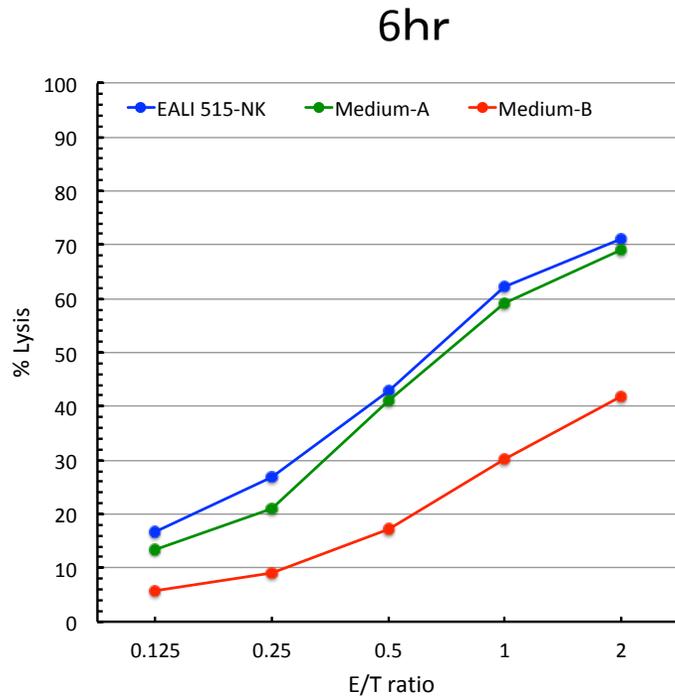
■ CD56+,CD3+
 ■ CD56+,CD3-
 ■ CD56-,CD3+
 ■ CD56-,CD3-



*** New medium EALI 515-NK support selective proliferation of NK cells**

Comparative study of cell killing activity by NK cells that were cultured in three media

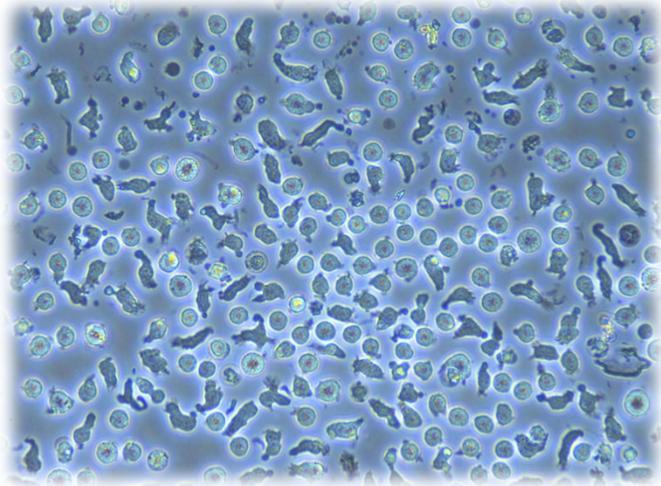
The cell killing activities against K562 Leukemia cell by NK cells which were cultured with 3 media were determined. K562 cell (as target) and 3 kinds NK cells (as effector) were mixed at indicated E/T ratio on Figure and co-cultured for 6 and 24 hr. After incubation, LDH enzyme activity of culture were measured by LDH Cyto-toxity Detection Kit (TaKaRa).



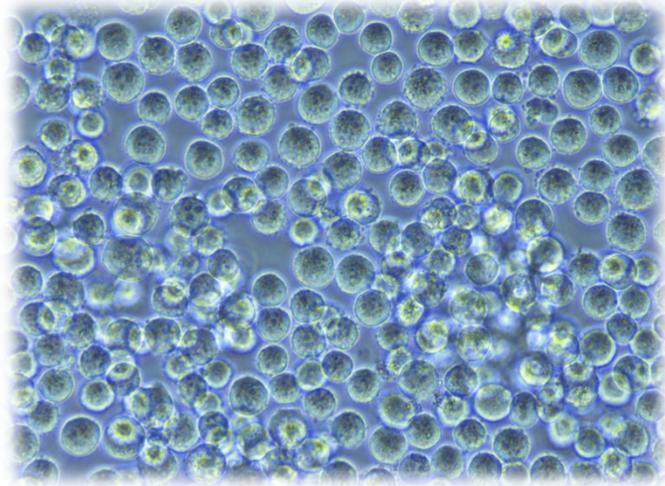
*** NK cells cultured with EALI 515-NK shown strongest tumor cell killing activity**

Photograph of Cell killing activity

CD56⁺/CD16⁺ NK cell

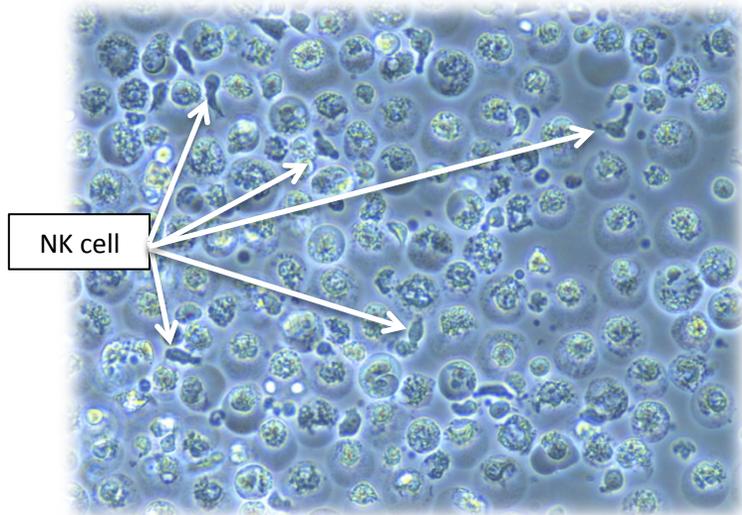


Leukemia cell K562



+

E/T ratio=0.5



E/T ratio=0.2

24hr

