Time: 2024.05.10-2024.05.29

1. **Experiment:** Cell proliferation assessment
2. **Time:** 2024.05.10-2024.05.29
3. **Member:** Xinyu Zhu, Song Zhang, Yinran Luo
4. **Material:** cell culture medium, growth curve plotting software
5. **Method:**
   (1) Sample Collection: At different days of cell culture (Day 1-Day 7), cell samples were collected from each culture flask. The samples were mixed thoroughly to ensure a uniform suspension.
   (2) Cell Counting: An aliquot of each cell sample was taken, and cells were counted using an automated cell counter. The total cell number was recorded for each time point.
   (3) Calculation of Fold Expansion: The fold expansion of cells was calculated by comparing the cell numbers at different time points to the initial cell count (Day 0). The fold increase was determined using the formula:
   (4) Plotting the Growth Curve: A cell growth curve was generated by plotting the fold expansion (y-axis) against the days of culture (x-axis). This curve was used to analyze the proliferation rate and growth kinetics of the cultured cells over time.

6. **Result:**

Table 1 Cell counting of control NK-92 under IL-2 stimulation

Number	control NK-92					
Day0	50000	50000	50000	50000	50000	50000
Day1	111,000	89,000	99,000	87,000	69,000	79,000
Day2	226,000	214,000	218,000	197,000	148,000	235,000
Day3	411,000	300,000	448,000	444,000	537,000	535,000
Day4	876,000	745,000	1,069,000	971,000	787,000	837,000
Day5	1,530,000	1,810,000	1,460,000	1,640,000	1,470,000	1,820,000
Day6	3,420,000	4,780,000	3,960,000	3,450,000	4,380,000	4,010,000
Day7	7,100,000	7,220,000	6,480,000	6,290,000	5,550,000	7,750,000

Table 2 Cell counting of control αCD19 CAR under IL-2 stimulation

Number	αCD19 CAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	106,000	103,000	112,000	115,000	90,000	85,000
Day2	205,000	205,000	249,000	188,000	172,000	156,000
Day3	420,000	403,000	514,000	377,000	429,000	428,000
Day4	716,000	774,000	867,000	1,021,000	852,000	733,000
Day5	1,720,000	1,750,000	1,350,000	1,910,000	1,750,000	1,660,000
Day6	3,740,000	3,230,000	3,840,000	3,820,000	3,640,000	3,730,000
Day7	7,180,000	6,760,000	6,260,000	5,410,000	7,520,000	6,160,000

Table 3 Cell counting of control αCD19 CAR-library under IL-2 stimulation

Number	αCD19 CAR-library					
Day0	50000	50000	50000	50000	50000	50000
Day1	98,000	103,000	94,000	93,000	109,000	126,000
Day2	260,000	166,000	224,000	177,000	246,000	207,000
Day3	459,000	442,000	395,000	315,000	404,000	344,000
Day4	984,000	747,000	496,000	881,000	611,000	1,000,000
Day5	1,140,000	1,360,000	1,460,000	1,550,000	1,180,000	1,430,000
Day6	3,510,000	2,520,000	3,720,000	3,390,000	3,190,000	1,770,000
Day7	7,600,000	6,430,000	9,010,000	6,550,000	5,010,000	4,890,000

Table 4 Cell counting of control NK-92 under Raji stimulation

Number	control NK-92					
Day0	50000	50000	50000	50000	50000	50000
Day1	20,100	37,400	27,000	34,600	29,600	38,900
Day2	12,400	18,100	22,000	16,400	19,400	18,200
Day3	6900	5550	5350	5550	4450	5700
Day4	0	0	0	1,100	1,900	400
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 5 Cell counting of control αCD19 CAR under Raji stimulation

Number	αCD19 CAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	21,800	21,300	15,600	19,400	21,500	16,100
Day2	12,300	12,100	11,800	11,700	10,600	12,400
Day3	2,640	2,470	2,330	3,070	2,190	2,370
Day4	0	0	0	0	0	0
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 6 Cell counting of control αCD19 CAR-library under Raji stimulation

Number	αCD19 CAR-library					
Day0	50000	50000	50000	50000	50000	50000
Day1	134,000	195,000	123,000	138,000	129,000	127,000
Day2	196,000	284,000	243,000	248,000	176,000	193,000
Day3	300,000	338,000	340,000	298,000	339,000	368,000
Day4	450,000	727,000	603,000	481,000	495,000	558,000
Day5	528,000	893,000	1,011,000	868,000	954,000	703,000
Day6	940,000	1,380,000	1,240,000	980,000	1,310,000	1,160,000
Day7	1,930,000	1,320,000	1,580,000	1,820,000	1,550,000	1,860,000

Table 7 Cell counting of NKR library cells under different tumor stimuli

Number	Raji			HepG2			AsPC1		
Day0	50000	50000	50000	50000	50000	50000	50000	50000	50000
Day1	76,000	87,000	83,000	94,000	69,000	80,000	77,000	76,000	81,000
Day2	134,000	128,000	157,000	149,000	159,000	137,000	122,000	96,000	132,000
Day3	229,000	194,000	221,000	290,000	270,000	307,000	216,000	214,000	194,000
Day4	265,000	429,000	340,000	516,000	377,000	430,000	300,000	375,000	241,000
Day5	473,000	592,000	665,000	613,000	665,000	786,000	397,000	397,000	705,000
Day6	1,009,000	1,170,000	898,000	1,347,000	1,450,000	1,077,000	947,000	897,000	730,000
Day7	1,490,000	1,461,000	1,544,000	2,080,000	2,039,000	2,460,000	1,457,000	1,321,000	1,635,000
Number	**A549**			**MCF7**			**PANC1**		
Day0	50000	50000	50000	50000	50000	50000	50000	50000	50000
Day1	97,000	80,000	88,000	76,000	63,000	89,000	73,000	79,000	83,000
Day2	148,000	99,000	126,000	98,000	108,000	93,000	97,000	124,000	110,000
Day3	284,000	246,000	194,000	155,000	174,000	146,000	275,000	201,000	240,000
Day4	373,000	302,000	451,000	265,000	340,000	258,000	372,000	359,000	388,000
Day5	654,000	674,000	750,000	230,000	487,000	352,000	553,000	673,000	528,000
Day6	885,000	830,000	1,213,000	872,000	351,000	452,000	1,070,000	861,000	1,002,000
Day7	1,797,000	2,653,000	1,362,000	941,000	1,349,000	1,181,000	977,000	1,440,000	1,618,000

Table 7 Cell counting of NK-92 cells under Raji stimuli

Number	NK-92					
Day0	50000	50000	50000	50000	50000	50000
Day1	33,900	29,400	34,100	36,300	26,900	23,900
Day2	11,500	10,180	8,980	10,430	8,710	8,800
Day3	2,460	2,930	2,580	3,760	2,650	3,310
Day4	950	830	1,020	830	930	1,140
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 8 Cell counting of αCD20 CAR cells under Raji stimuli

	αCD20 CAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	30,900	23,800	27,000	25,800	24,000	26,100
Day2	8,480	9,570	7,590	8,240	8,620	10,090
Day3	1,710	2,020	1,580	1,590	1,930	2,140
Day4	1,810	1,920	1,550	1,470	2,020	1,890
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 9 Cell counting of αCD20 eCAR cells under Raji stimuli

	αCD20 eCAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	86,200	79,200	70,700	74,100	95,800	100,700
Day2	126,000	116,000	135,000	87,000	130,000	107,000
Day3	162,000	184,000	161,000	154,000	128,000	196,000
Day4	201,000	259,000	336,000	209,000	255,000	240,000
Day5	299,000	432,000	484,000	412,000	435,000	466,000
Day6	653,000	520,000	705,000	572,000	597,000	711,000
Day7	759,000	872,000	1,057,000	835,000	800,000	910,000

Table 10 Cell counting of NK-92 cells under HepG2 stimuli

Number	NK-92					
Day0	50000	50000	50000	50000	50000	50000
Day1	29,300	25,500	29,400	30,900	27,000	28,500
Day2	15,800	19,500	13,500	21,600	16,400	17,300
Day3	3,670	4,100	3,170	3,070	3,880	3,160
Day4	0	1,860	0	0	1,510	0
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 11 Cell counting of αGPC3 CAR cells under HepG2 stimuli

Number	αGPC3 CAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	18,600	17,600	16,500	19,000	13,200	15,600
Day2	4,170	5,590	5,650	6,680	7,020	4,810
Day3	930	910	780	1,120	920	1,110
Day4	0	0	0	0	0	0
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 12 Cell counting of αGPC3 eCAR cells under HepG2 stimuli

Number	αGPC3 eCAR					
Day0	50000	50000	50000	50000	50000	50000
Day1	68,900	69,600	88,900	88,100	81,600	101,300
Day2	135,000	123,000	146,000	143,000	135,000	122,000
Day3	166,000	232,000	175,000	238,000	220,000	249,000
Day4	248,000	347,000	221,000	315,000	322,000	467,000
Day5	484,000	578,000	497,000	552,000	716,000	596,000

Day6	635,000	763,000	862,000	660,000	1,044,000	928,000
Day7	1,340,000	1,440,000	1,850,000	1,470,000	1,550,000	1,280,000

Table 13 Cell counting of NK-92 cells under AsPC-1 stimuli

Number	**NK-92**					
Day0	50000	50000	50000	50000	50000	50000
Day1	22,000	22,800	22,800	22,900	25,200	26,400
Day2	12,610	8,190	9,870	8,260	7,620	11,140
Day3	3,630	5,480	4,530	4,890	3,740	3,040
Day4	820	800	670	840	700	650
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 14 Cell counting of αMSLN CAR cells under AsPC-1 stimuli

Number	**αMSLN CAR**					
Day0	50000	50000	50000	50000	50000	50000
Day1	20,100	27,600	20,300	24,300	25,200	25,300
Day2	10,920	9,780	10,290	9,630	7,210	11,810
Day3	2,170	1,700	1,530	2,070	1,730	2,050
Day4	0	0	0	350	270	0
Day5	0	0	0	0	0	0
Day6	0	0	0	0	0	0
Day7	0	0	0	0	0	0

Table 15 Cell counting of αMSLN eCAR cells under AsPC-1 stimuli

Number	**αMSLN eCAR**					
Day0	50000	50000	50000	50000	50000	50000
Day1	63,700	82,500	89,100	105,100	73,200	81,600
Day2	125,000	159,000	136,000	145,000	165,000	139,000
Day3	263,000	266,000	234,000	183,000	267,000	206,000
Day4	441,000	422,000	413,000	486,000	475,000	377,000
Day5	541,000	749,000	548,000	777,000	991,000	782,000
Day6	1,340,000	1,010,000	1,360,000	1,050,000	1,410,000	1,050,000
Day7	1,910,000	2,190,000	1,660,000	2,120,000	1,920,000	2,390,000