Figure 1

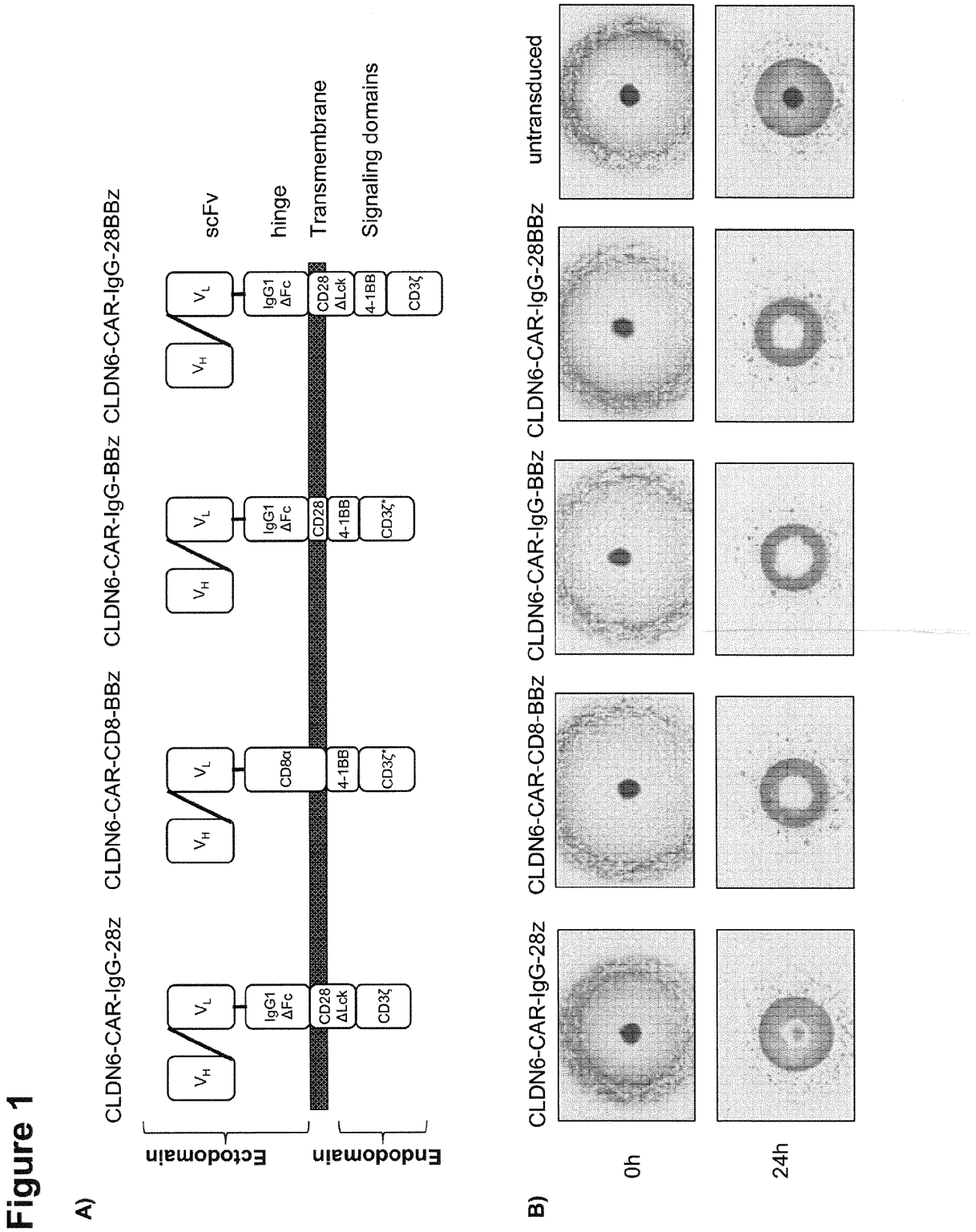

A)
CLDN6-CAR-IgG-28z
CLDN6-CAR-CD8-BBz
CLDN6-CAR-IgG-BBz
CLDN6-CAR-IgG-28BBz
scFv
hinge
Transmembrane
Signaling domains
Ectodomain
Endodomain
V_H
V_L
IgG1 ΔFc
CD8α
CD28
CD28 ΔLck
4-1BB
CD3ζ
CD3ζ*
B)
CLDN6-CAR-IgG-28z
CLDN6-CAR-CD8-BBz
CLDN6-CAR-IgG-BBz
CLDN6-CAR-IgG-28BBz
untransduced
0h
24h

Figure 2

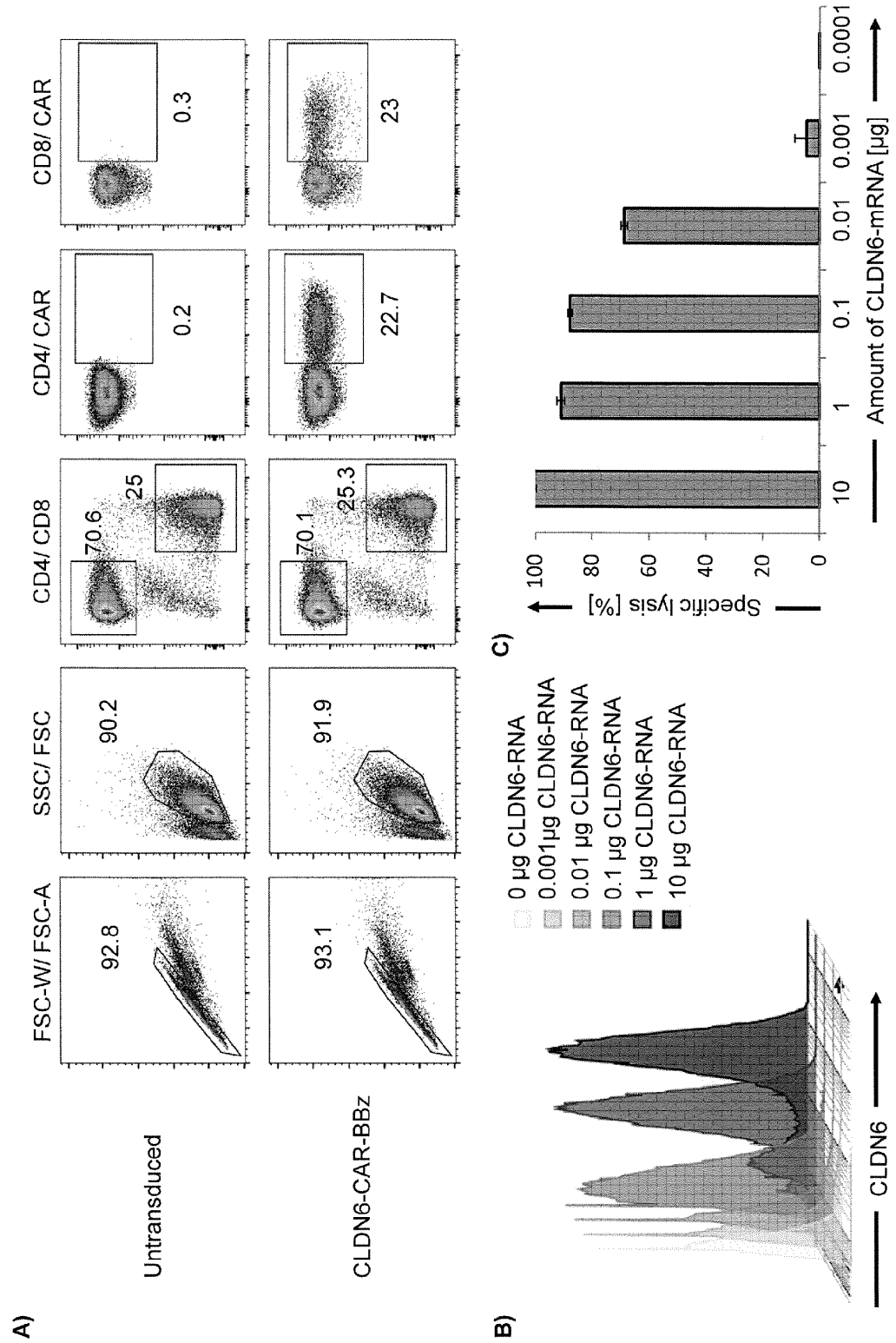
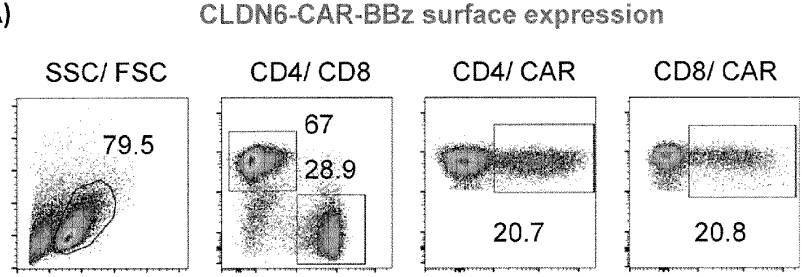

A)
FSC-W/ FSC-A
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
Untransduced
CLDN6-CAR-BBz
92.8
90.2
70.6
25
0.2
0.3
93.1
91.9
70.1
25.3
22.7
23
B)
0 µg CLDN6-RNA
0.001µg CLDN6-RNA
0.01 µg CLDN6-RNA
0.1 µg CLDN6-RNA
1 µg CLDN6-RNA
10 µg CLDN6-RNA
CLDN6
C)
Specific lysis [%]
100
80
60
40
20
0
10
1
0.1
0.01
0.001
0.0001
Amount of CLDN6-mRNA [µg]

Figure 3

A)



B)

CLDN6+ and CLDN6- tumor cell lines

	Name	CLDN6 pos/neg	Origin
1	LCLC-103H	negative	Lung
2	Mel-526	negative	Skin
3	SK-MEL-37	negative	Skin
4	SK-OV-3	negative	Ovary
5	23132-87	negative	Gastic
6	Colo-699-N	negative	Lung
7	HEK-293	negative	Embryonic kidney
8	MDA-MB-231	negative	Breast
9	PA-1-SC12	positive	Ovary
10	NIH-OvCar-3	positive	Ovary
11	JAR	positive	Embryo
12	NEC-8	positive	Embryo

Figure 3

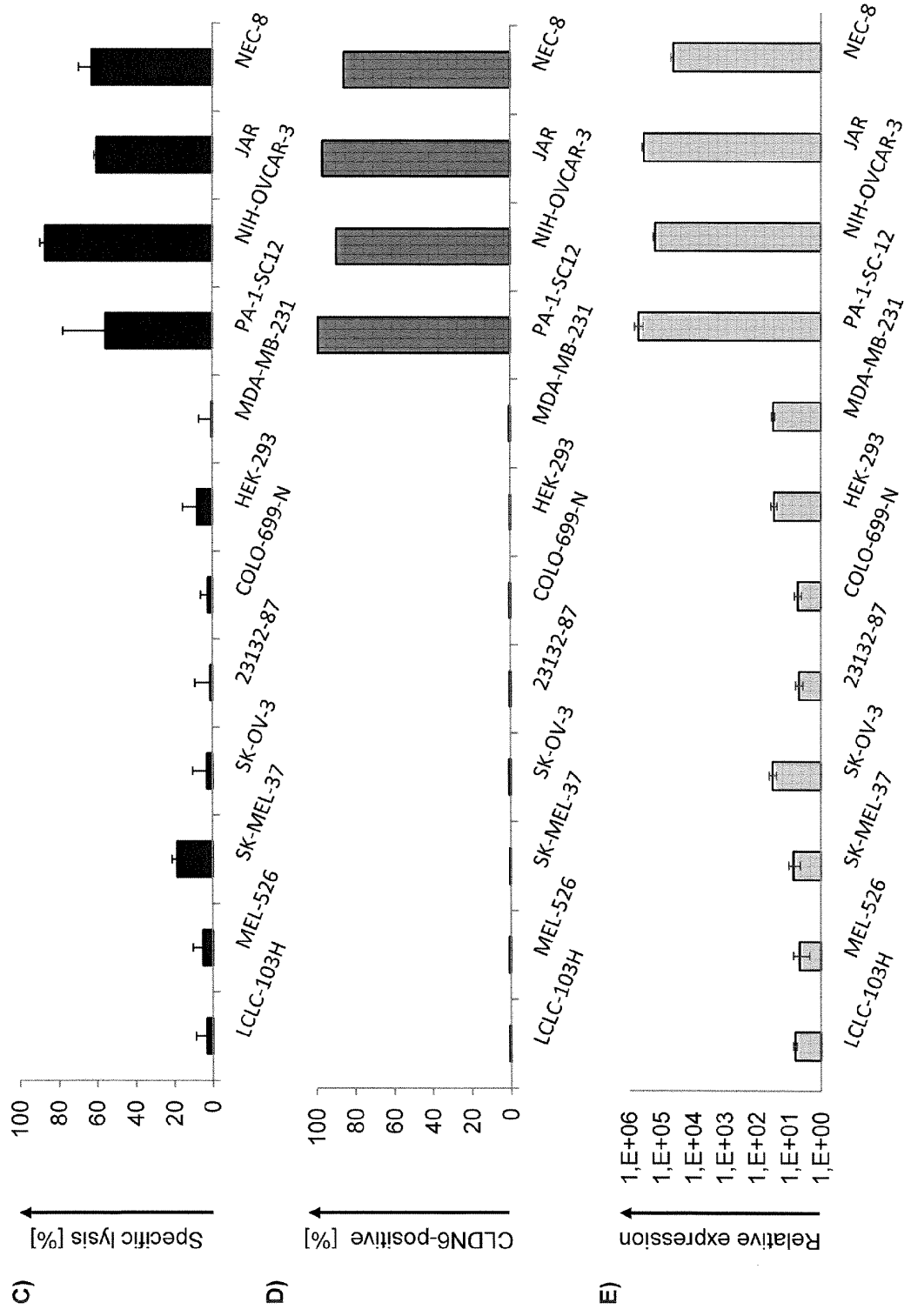

C)
Specific lysis [%]
100
80
60
40
20
0
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC12
NIH-OVCAR-3
JAR
NEC-8
D)
CLDN6-positive [%]
100
80
60
40
20
0
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC12
NIH-OVCAR-3
JAR
NEC-8
E)
Relative expression
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
LCLC-103H
MEL-526
SK-MEL-37
SK-OV-3
23132-87
COLO-699-N
HEK-293
MDA-MB-231
PA-1-SC12
NIH-OVCAR-3
JAR
NEC-8

Figure 4

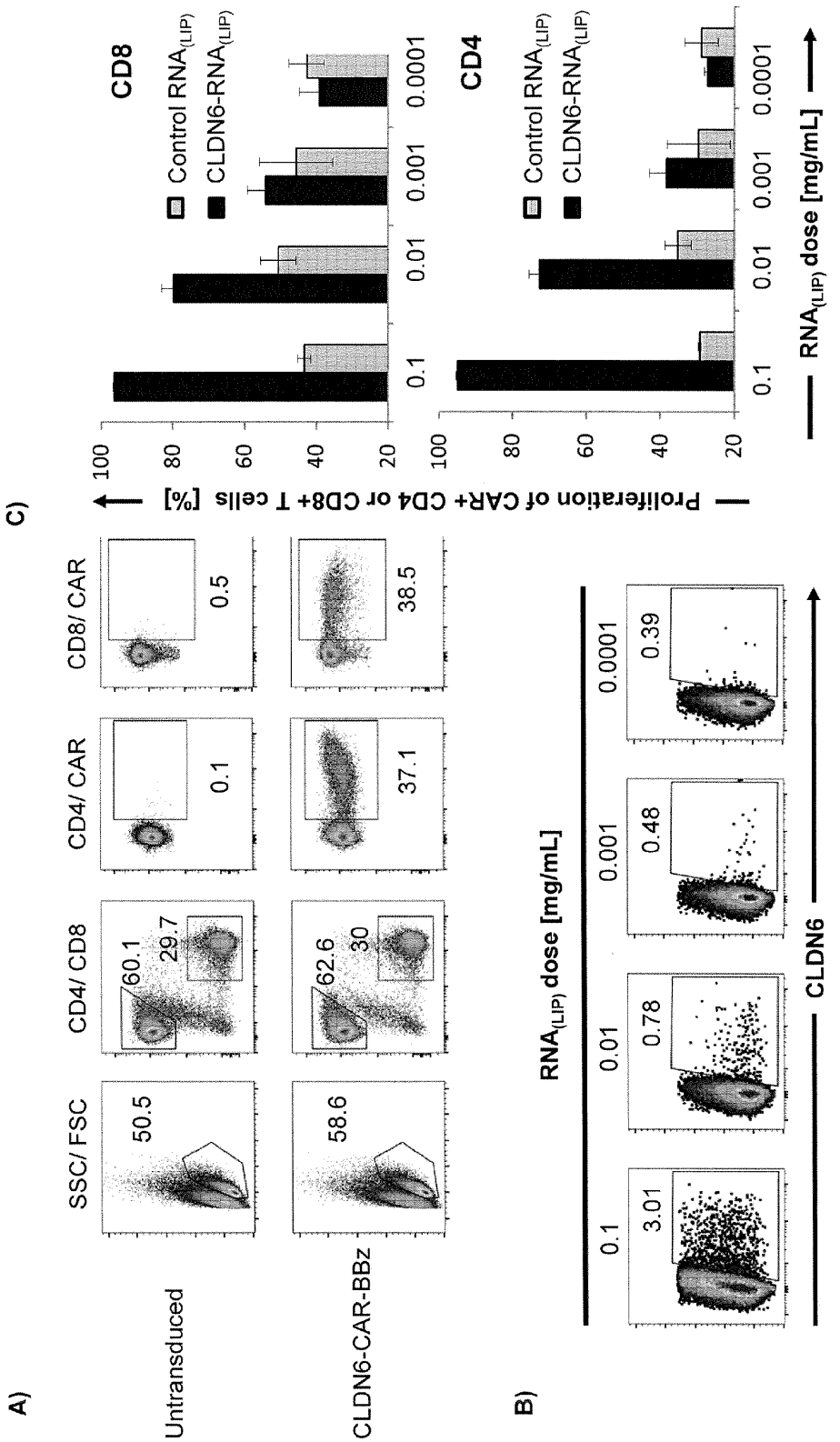

A)
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
Untransduced
CLDN6-CAR-BBz
50.5
60.1
29.7
0.1
0.5
58.6
62.6
30
37.1
38.5
B)
RNA(LIP) dose [mg/mL]
0.1
0.01
0.001
0.0001
3.01
0.78
0.48
0.39
CLDN6
C)
Proliferation of CAR+ CD4 or CD8+ T cells [%]
CD8
CD4
Control RNA(LIP)
CLDN6-RNA(LIP)
RNA(LIP) dose [mg/mL]

Figure 5

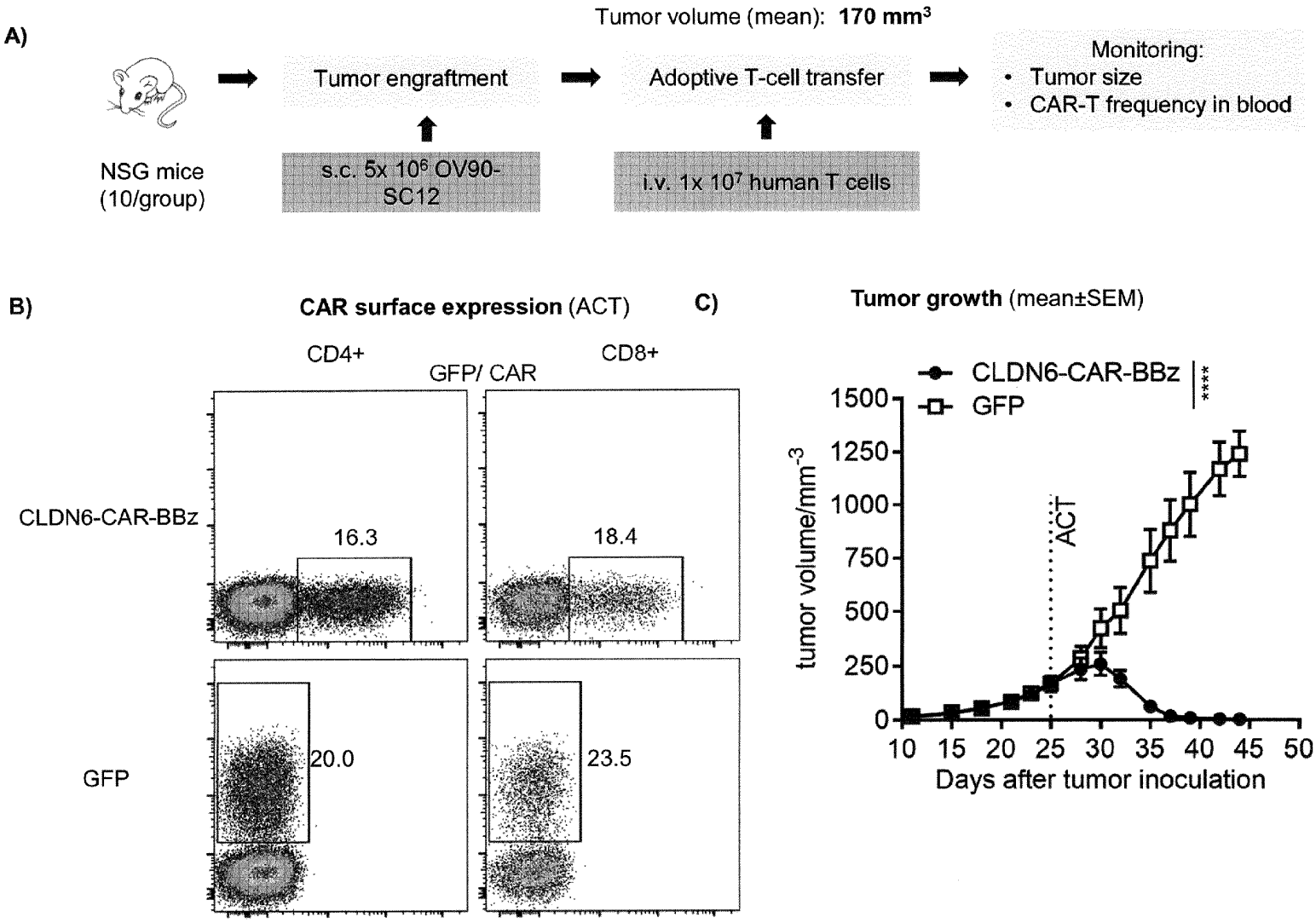

A)
Tumor volume (mean): 170 mm3
Tumor engraftment
Adoptive T-cell transfer
Monitoring:
• Tumor size
• CAR-T frequency in blood
NSG mice
(10/group)
s.c. 5x 10^6 OV90-SC12
i.v. 1x 10^7 human T cells
B)
CAR surface expression (ACT)
CD4+
GFP/ CAR
CD8+
CLDN6-CAR-BBz
GFP
16.3
18.4
20.0
23.5
C)
Tumor growth (mean±SEM)
CLDN6-CAR-BBz
GFP
****
tumor volume/mm-3
ACT
Days after tumor inoculation

Figure 5

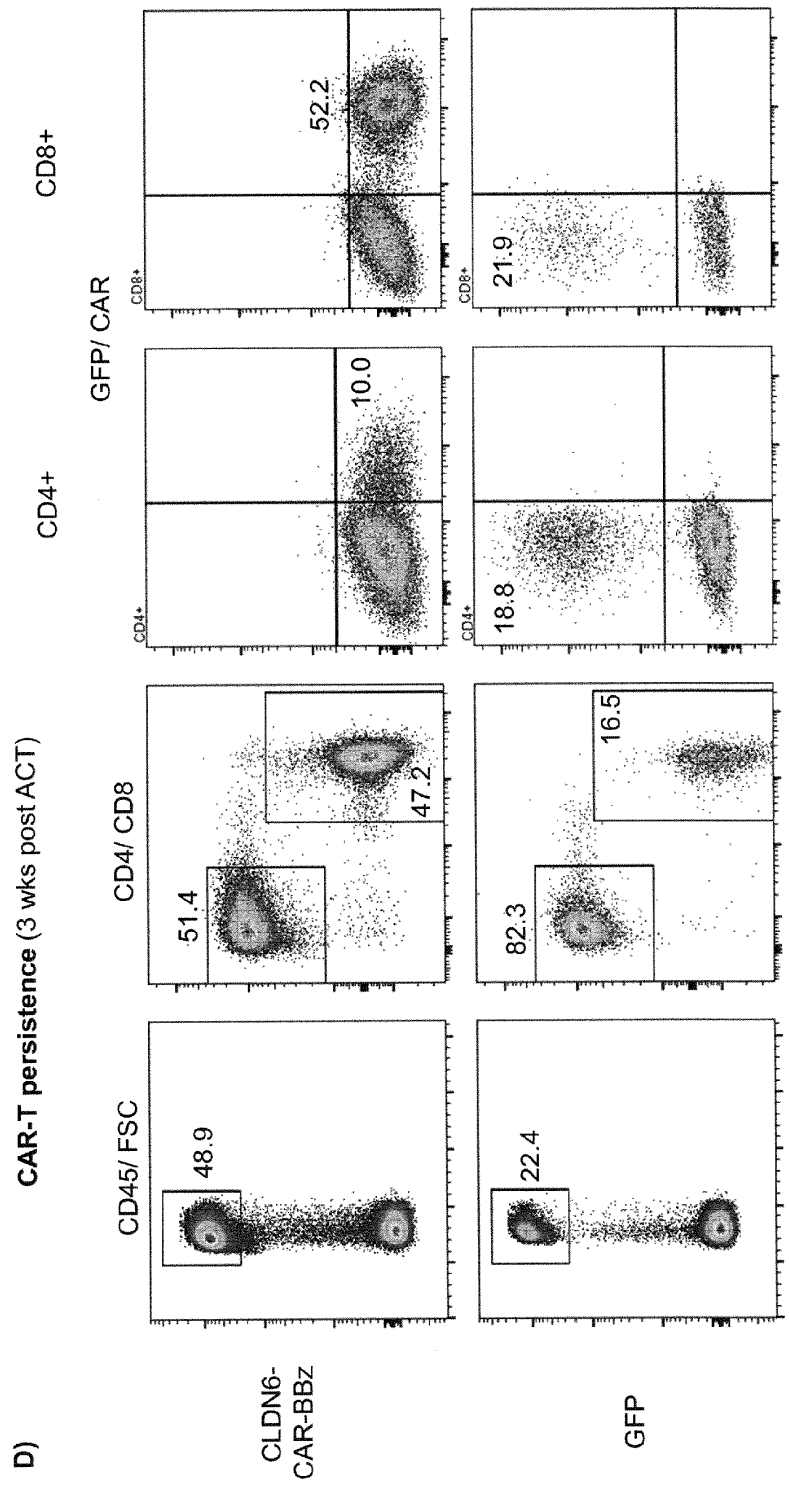

D)
CAR-T persistence (3 wks post ACT)
CD4+
CD8+
GFP/ CAR
CD4/ CD8
CD45/ FSC
CLDN6-CAR-BBz
GFP
48.9
51.4
47.2
10.0
52.2
22.4
82.3
16.5
18.8
21.9

Figure 6

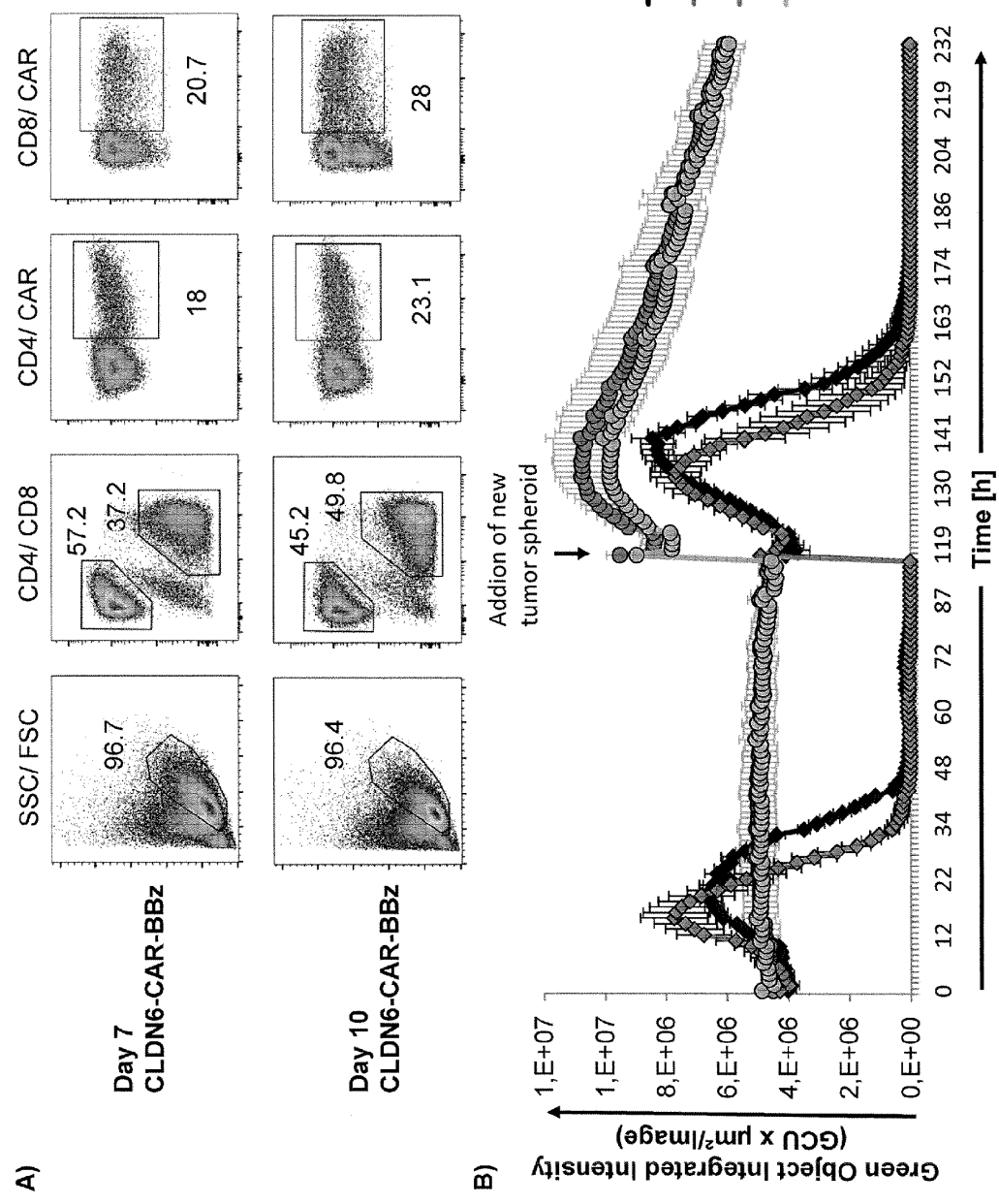

A)
SSC/ FSC
CD4/ CD8
CD4/ CAR
CD8/ CAR
96.7
57.2
37.2
18
20.7
Day 7
CLDN6-CAR-BBz
96.4
45.2
49.8
23.1
28
Day 10
CLDN6-CAR-BBz
B)
Addion of new
tumor spheroid
CLDN6-CAR-BBz d7
untransduced d7
CLDN6-CAR-BBz d10
untransduced d10
Green Object Integrated Intensity
(GCU x µm²/Image)
1,E+07
1,E+07
8,E+06
6,E+06
4,E+06
2,E+06
0,E+00
0 12 22 34 48 60 72 87 119 130 141 152 163 174 186 204 219 232
Time [h]

Figure 7

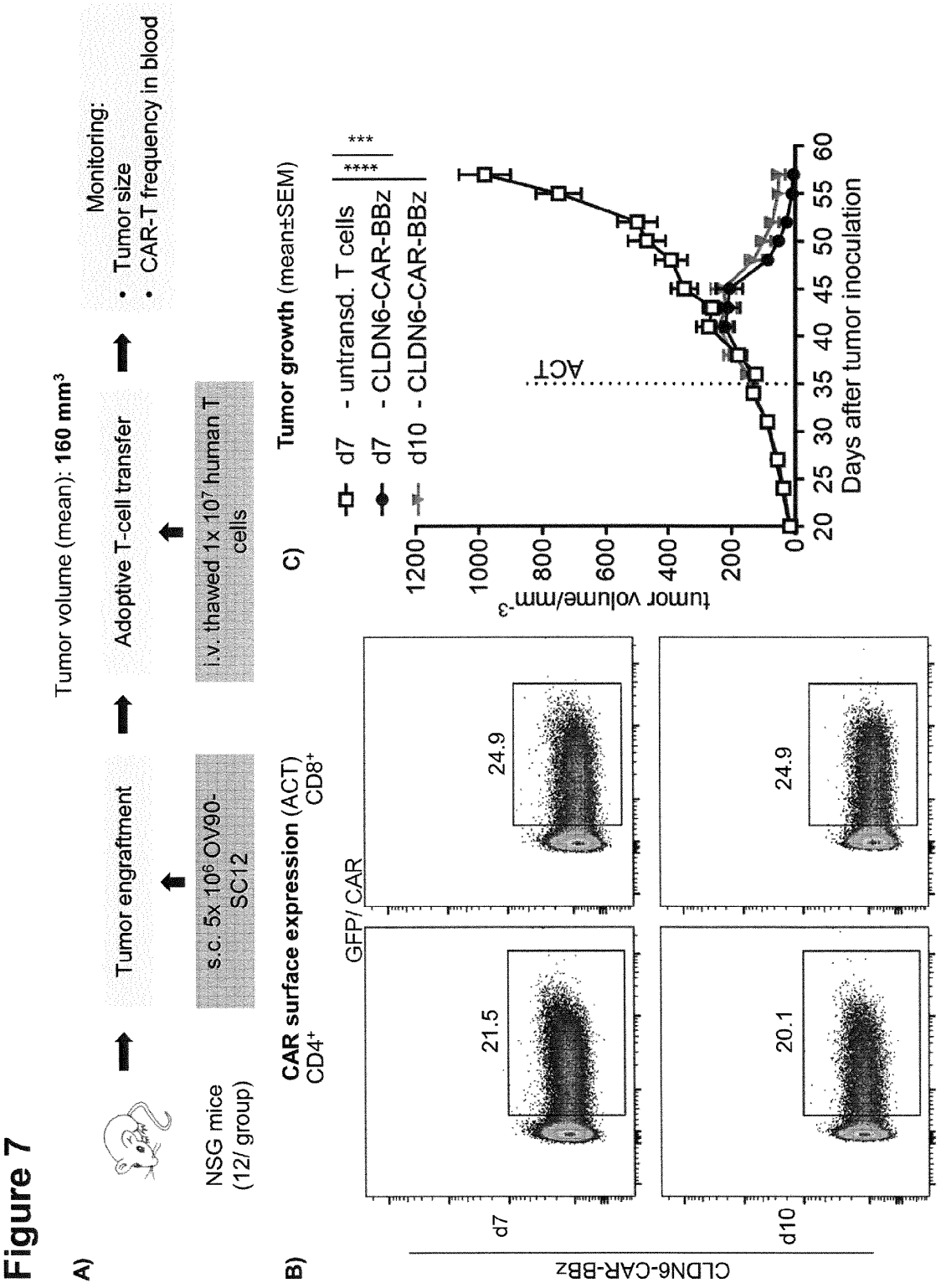

A)
Tumor volume (mean): 160 mm3
NSG mice (12/ group)
Tumor engraftment
s.c. 5x 10^6 OV90-SC12
Adoptive T-cell transfer
i.v. thawed 1x 10^7 human T cells
Monitoring:
• Tumor size
• CAR-T frequency in blood
B)
CAR surface expression (ACT)
CD4+
CD8+
GFP/ CAR
CLDN6-CAR-BBz
d7
d10
21.5
24.9
20.1
24.9
C)
Tumor growth (mean±SEM)
d7 - untransd. T cells
d7 - CLDN6-CAR-BBz
d10 - CLDN6-CAR-BBz
***
****
ACT
tumor volume/mm-3
Days after tumor inoculation

Figure 7

D)

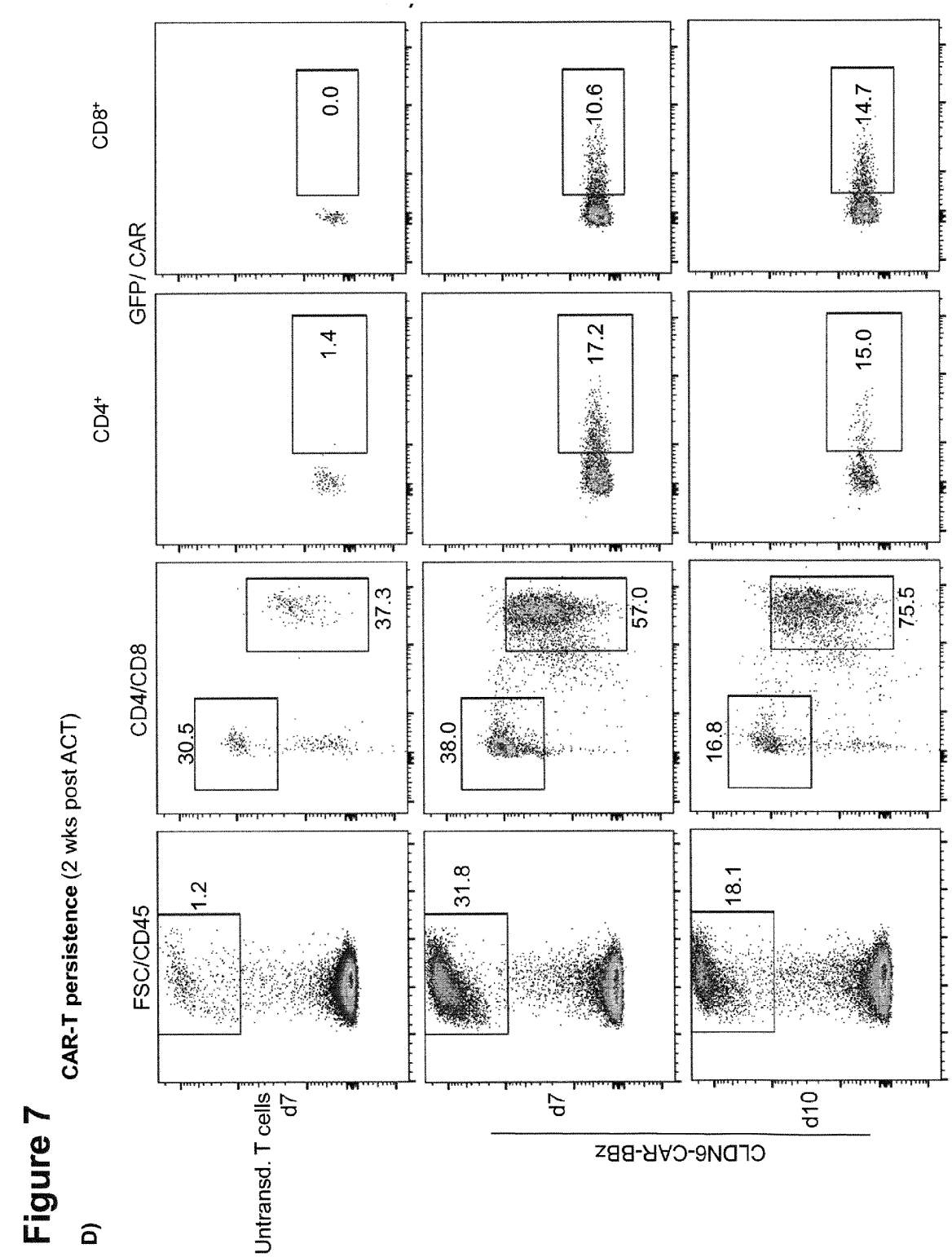

CAR-T persistence (2 wks post ACT)
FSC/CD45
CD4/CD8
CD4+
GFP/ CAR
CD8+
Untransd. T cells
d7
CLDN6-CAR-BBz
d7
d10
1.2
30.5
37.3
1.4
0.0
31.8
38.0
57.0
17.2
10.6
18.1
16.8
75.5
15.0
14.7

Figure 8

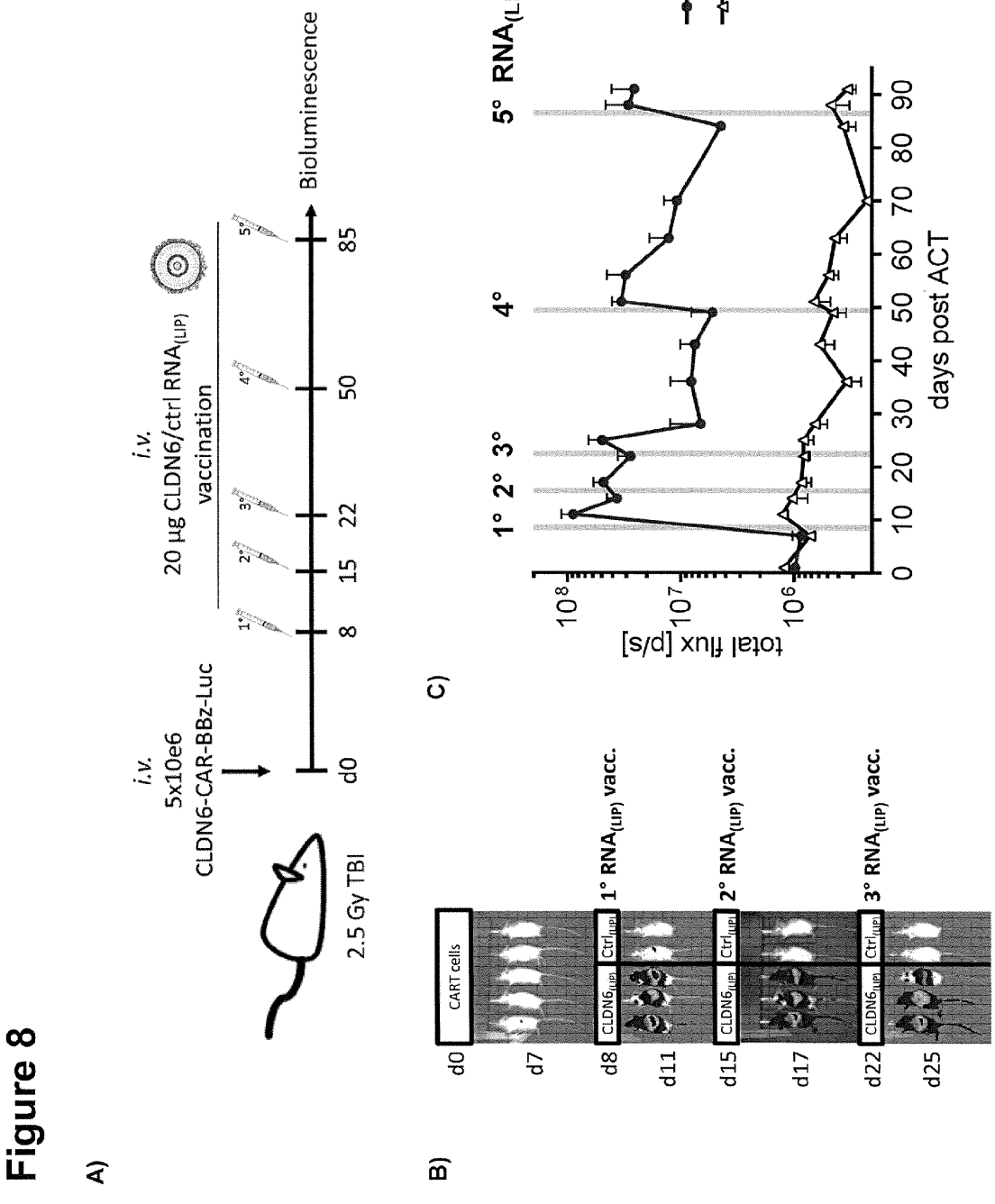
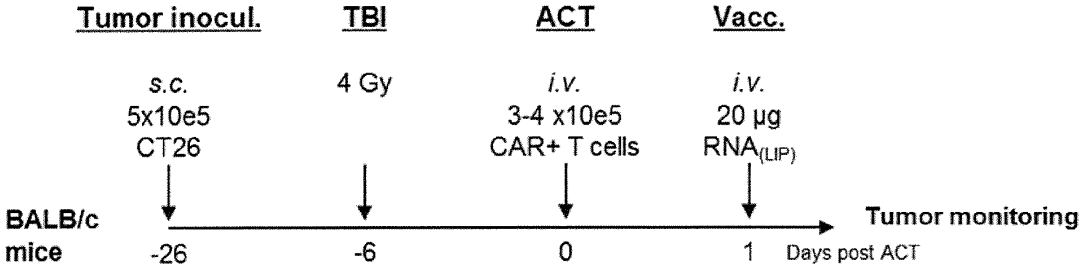

A)
i.v.
5x10e6
CLDN6-CAR-BBz-Luc
2.5 Gy TBI
d0
8
15
22
50
85
Bioluminescence
i.v.
20 µg CLDN6/ctrl RNA(LIP)
vaccination
1°
2°
3°
4°
5°
B)
CART cells
CLDN6(LIP)
Ctrl(LIP)
1° RNA(LIP) vacc.
2° RNA(LIP) vacc.
3° RNA(LIP) vacc.
d0
d7
d8
d11
d15
d17
d22
d25
C)
5° RNA(LIP)vaccination
1° 2° 3° 4°
CLDN6 RNA(LIP)
ctrl RNA(LIP)
total flux [p/s]
days post ACT

Figure 9

A)


Tumor inocul.
TBI
ACT
Vacc.
s.c.
5x10e5
CT26
4 Gy
i.v.
3-4 x10e5
CAR+ T cells
i.v.
20 µg
RNA(LIP)
BALB/c
mice
-26
-6
0
1
Days post ACT
Tumor monitoring

B)

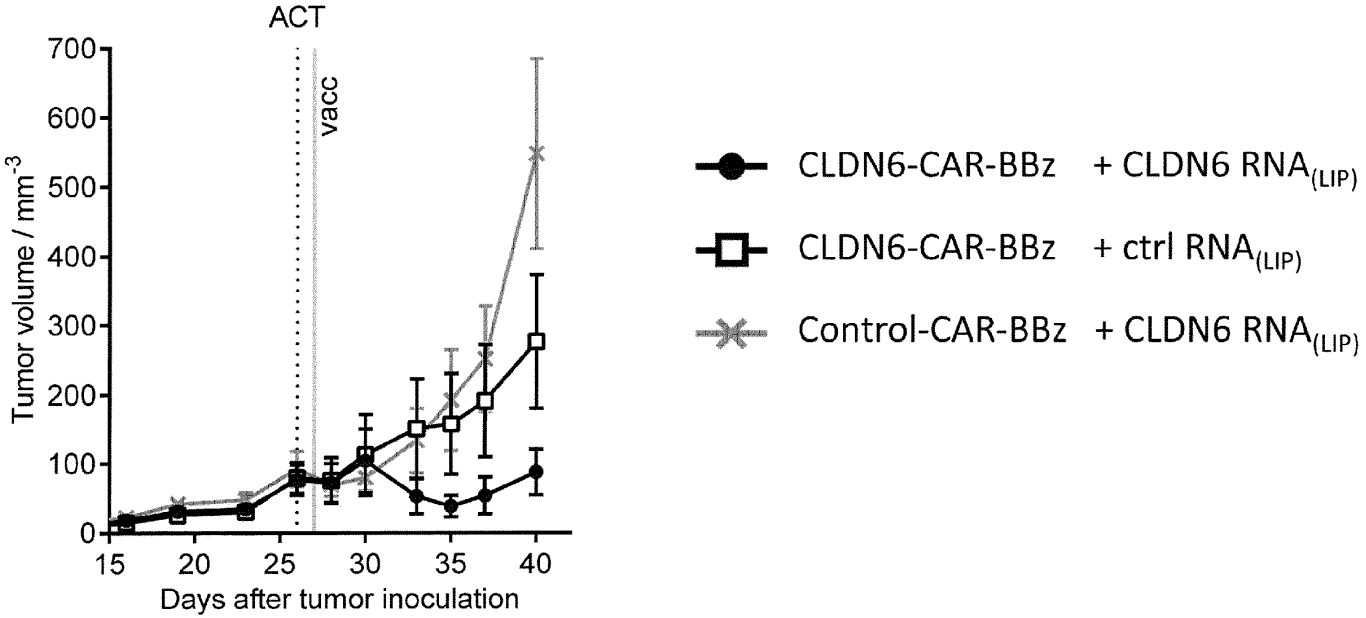

ACT
vacc
Tumor volume / mm-3
700
600
500
400
300
200
100
0
15
20
25
30
35
40
Days after tumor inoculation
CLDN6-CAR-BBz + CLDN6 RNA(LIP)
CLDN6-CAR-BBz + ctrl RNA(LIP)
Control-CAR-BBz + CLDN6 RNA(LIP)

Figure 10

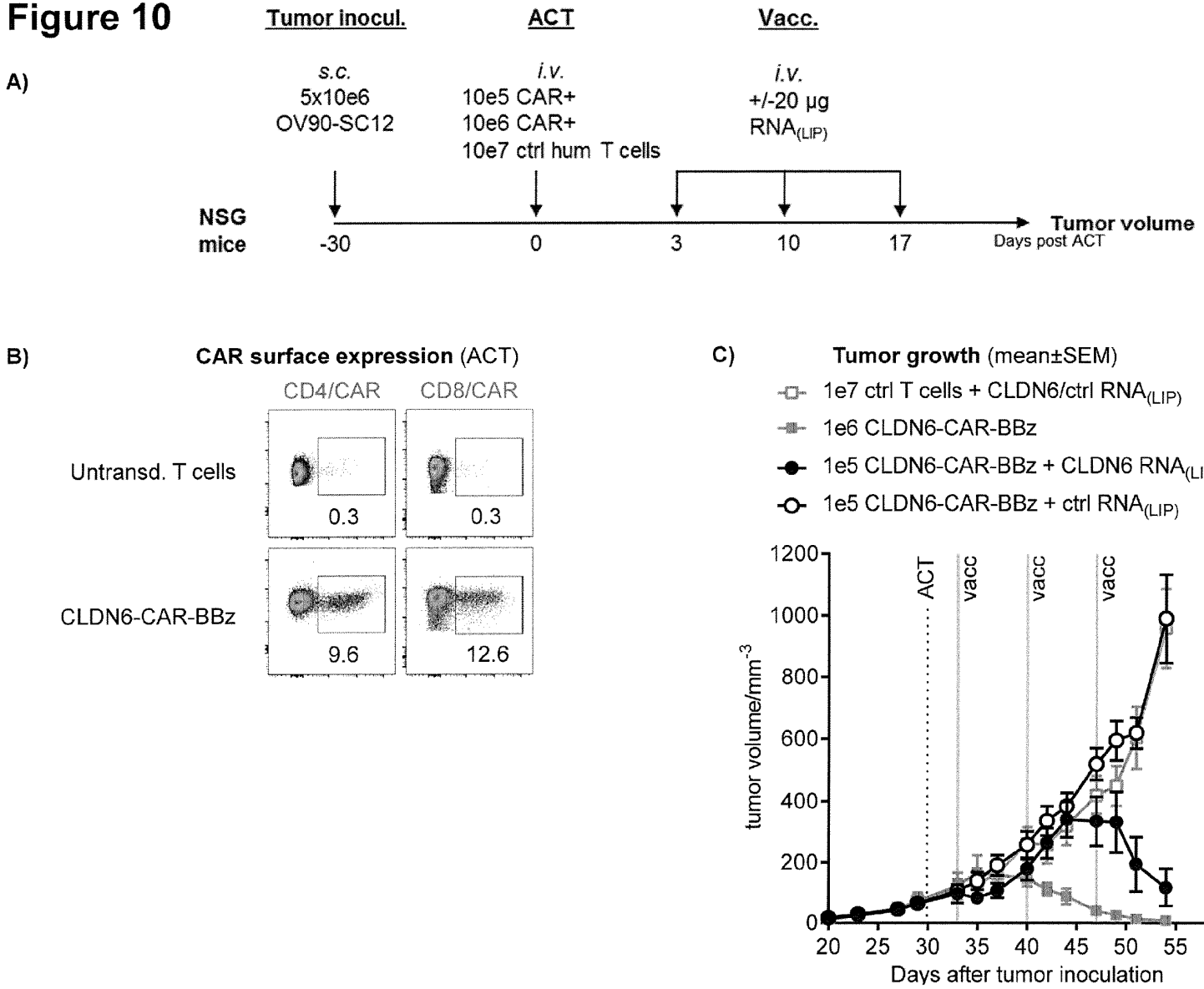

A)
Tumor inocul.
ACT
Vacc.
s.c.
5x10e6
OV90-SC12
i.v.
10e5 CAR+
10e6 CAR+
10e7 ctrl hum T cells
i.v.
+/-20 µg
RNA(LIP)
NSG
mice
-30
0
3
10
17
Tumor volume
Days post ACT
B)
CAR surface expression (ACT)
CD4/CAR
CD8/CAR
Untransd. T cells
CLDN6-CAR-BBz
0.3
0.3
9.6
12.6
C)
Tumor growth (mean±SEM)
1e7 ctrl T cells + CLDN6/ctrl RNA(LIP)
1e6 CLDN6-CAR-BBz
1e5 CLDN6-CAR-BBz + CLDN6 RNA(LIP)
1e5 CLDN6-CAR-BBz + ctrl RNA(LIP)
***
ACT
vacc
vacc
vacc
tumor volume/mm-3
0
200
400
600
800
1000
1200
20
25
30
35
40
45
50
55
60
Days after tumor inoculation

Figure 10

D)

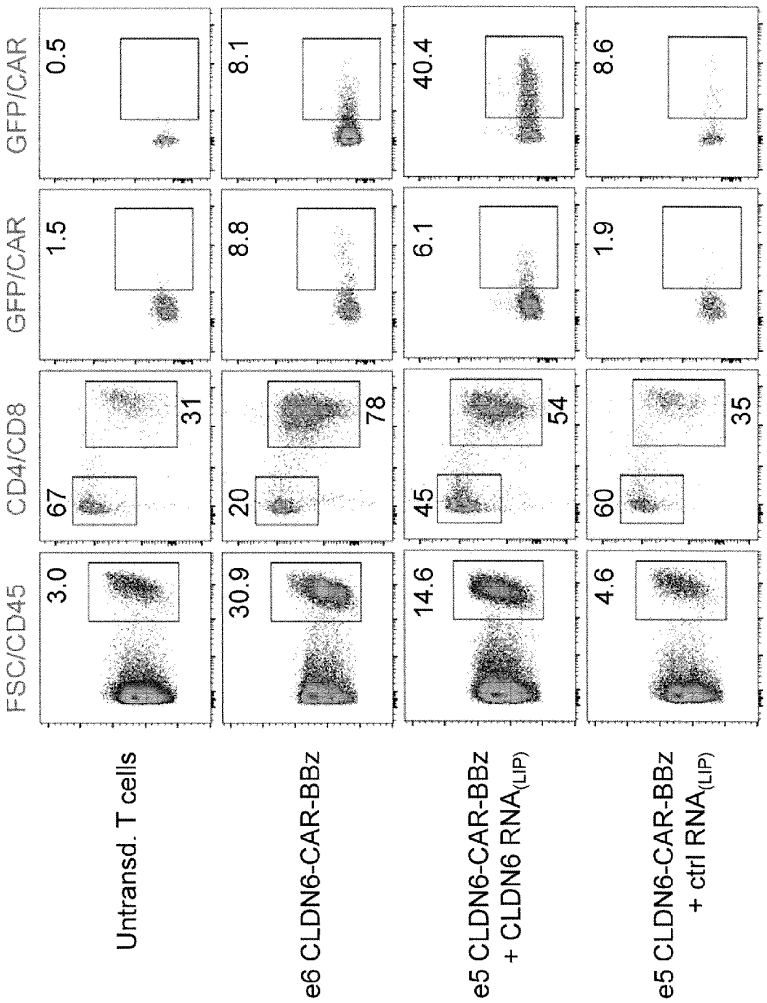

CAR-T persistence (2.5 wks post ACT)
FSC/CD45
CD4/CD8
CD4+ GFP/CAR
CD8+ GFP/CAR
3.0
67
31
1.5
0.5
30.9
20
78
8.8
8.1
14.6
45
54
6.1
40.4
4.6
60
35
1.9
8.6
Untransd. T cells
1e6 CLDN6-CAR-BBz
1e5 CLDN6-CAR-BBz + CLDN6 RNA(LIP)
1e5 CLDN6-CAR-BBz + ctrl RNA(LIP)

Figure 11

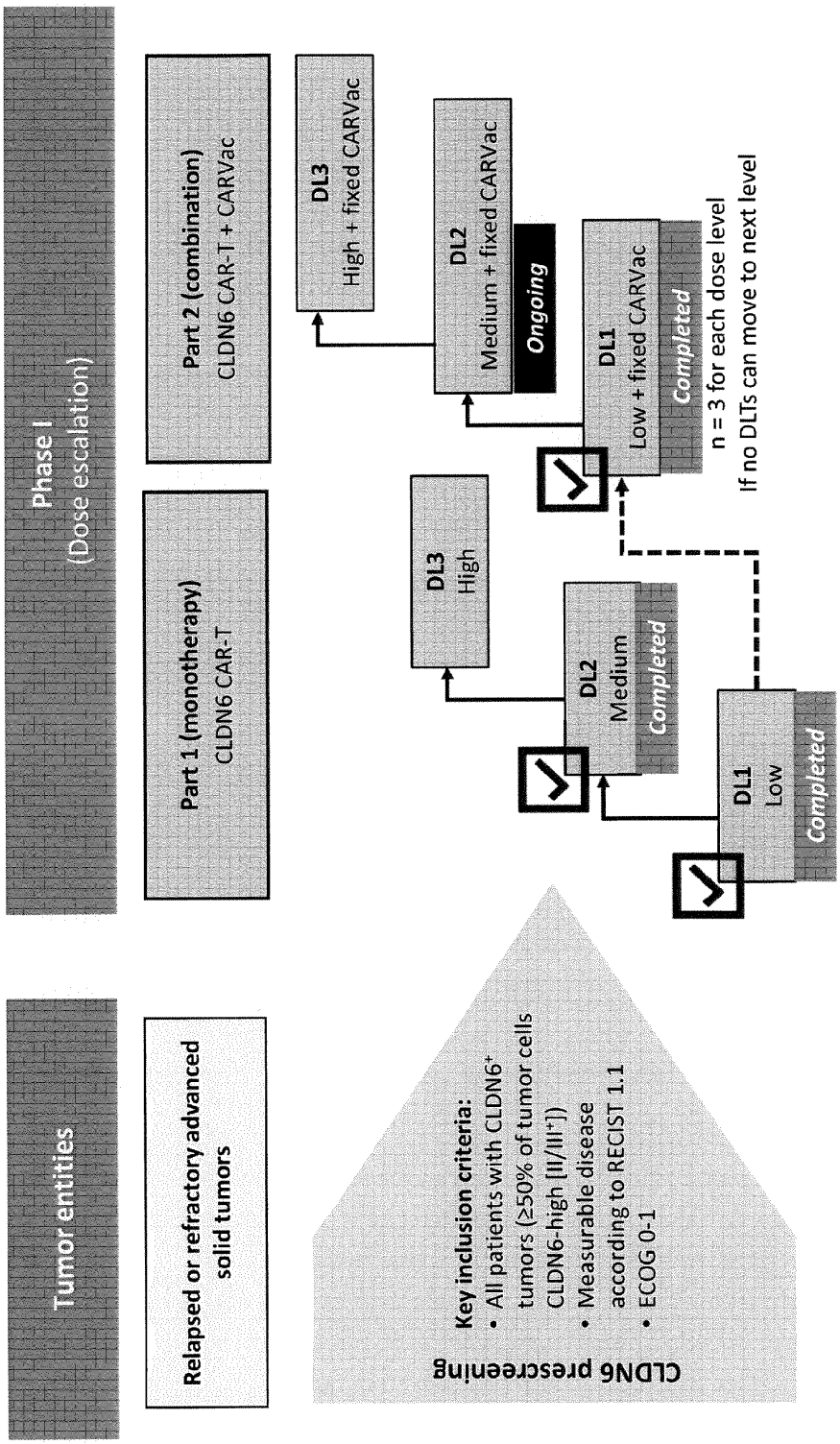

Tumor entities
Relapsed or refractory advanced solid tumors
CLDN6 prescreening
Key inclusion criteria:
• All patients with CLDN6+ tumors (≥50% of tumor cells CLDN6-high [II/III+])
• Measurable disease according to RECIST 1.1
• ECOG 0-1
Phase I
(Dose escalation)
Part 1 (monotherapy)
CLDN6 CAR-T
Part 2 (combination)
CLDN6 CAR-T + CARVac
DL1
Low
Completed
DL2
Medium
Completed
DL3
High
DL1
Low + fixed CARVac
Completed
DL2
Medium + fixed CARVac
Ongoing
DL3
High + fixed CARVac
n = 3 for each dose level
If no DLTs can move to next level

Figure 12

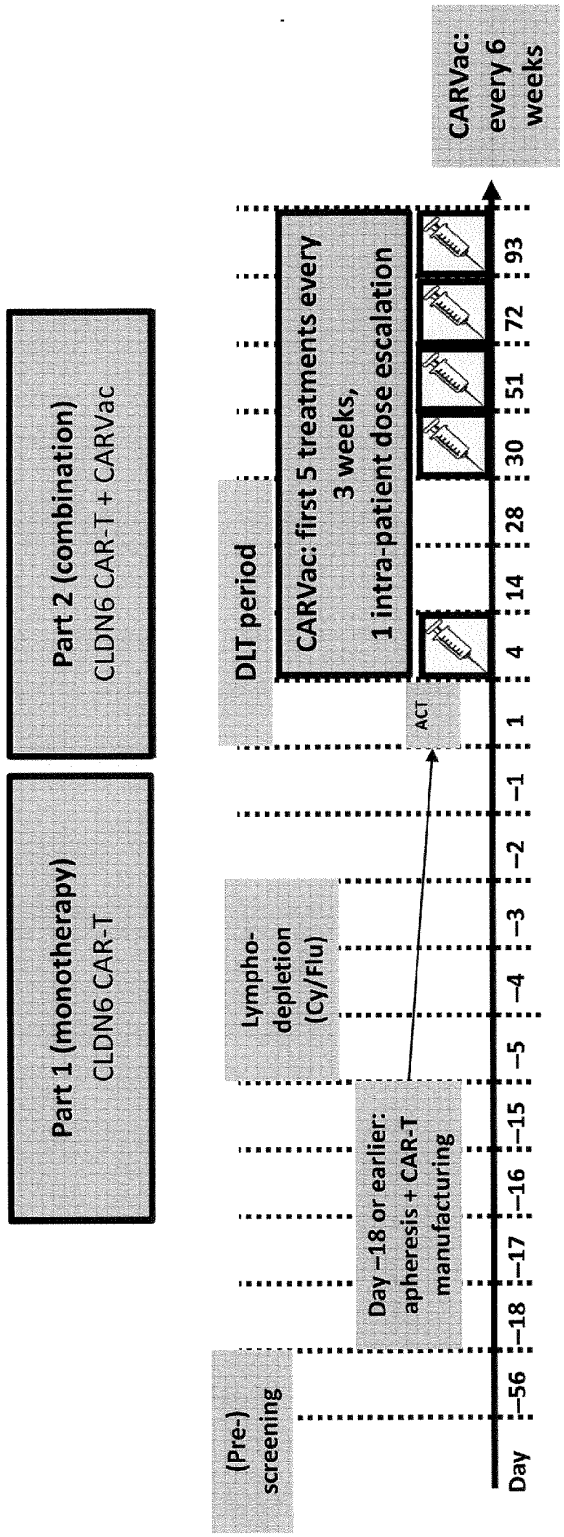
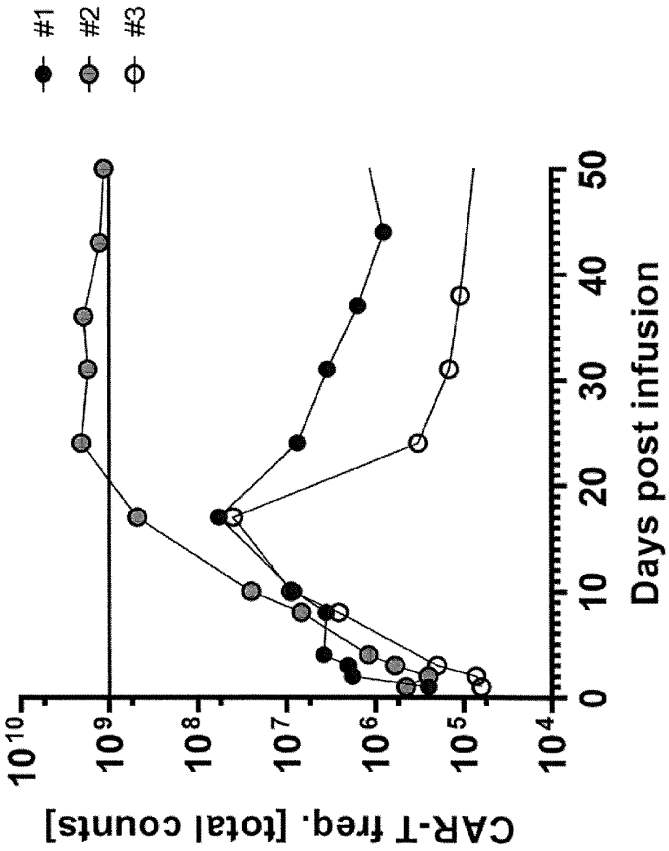

Part 1 (monotherapy)
CLDN6 CAR-T
Part 2 (combination)
CLDN6 CAR-T + CARVac
(Pre-) screening
Day –18 or earlier: apheresis + CAR-T manufacturing
Lympho-depletion (Cy/Flu)
DLT period
CARVac: first 5 treatments every 3 weeks, 1 intra-patient dose escalation
CARVac: every 6 weeks
ACT
Day
–56
–18
–17
–16
–15
–5
–4
–3
–2
–1
1
4
14
28
30
51
72
93

Figure 13A


#1
#2
#3
CAR-T freq. [total counts]
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
0
10
20
30
40
50
Days post infusion

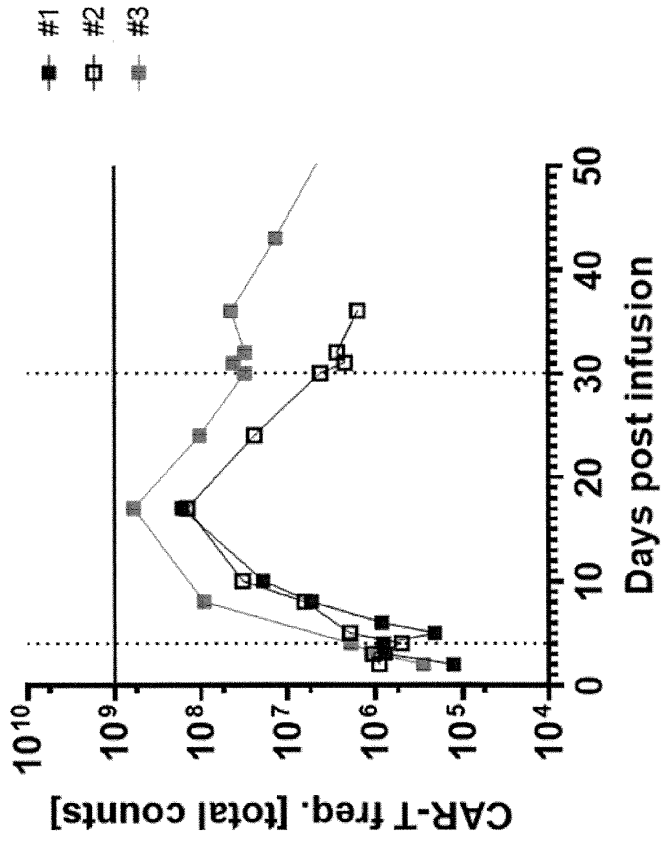

#1
#2
#3
CAR-T freq. [total counts]
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
0
10
20
30
40
50
Days post infusion

Figure 13B

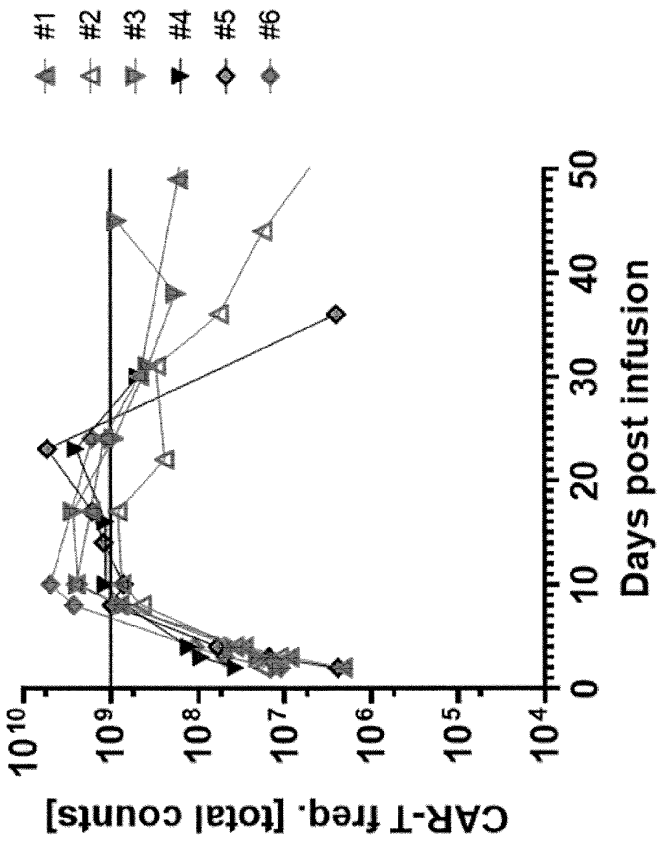

#1
#2
#3
#4
#5
#6
CAR-T freq. [total counts]
10^10
10^9
10^8
10^7
10^6
10^5
10^4
0
10
20
30
40
50
Days post infusion

Figure 13C

Figure 14

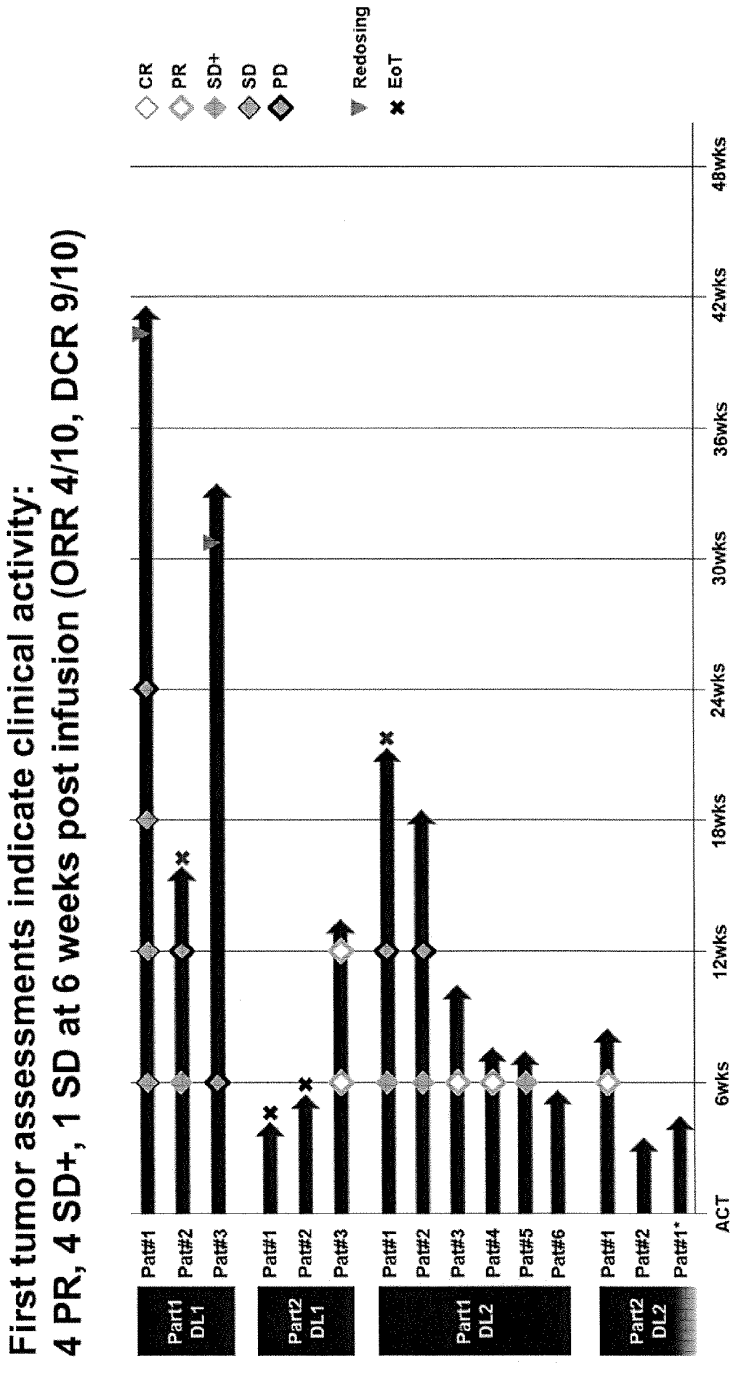
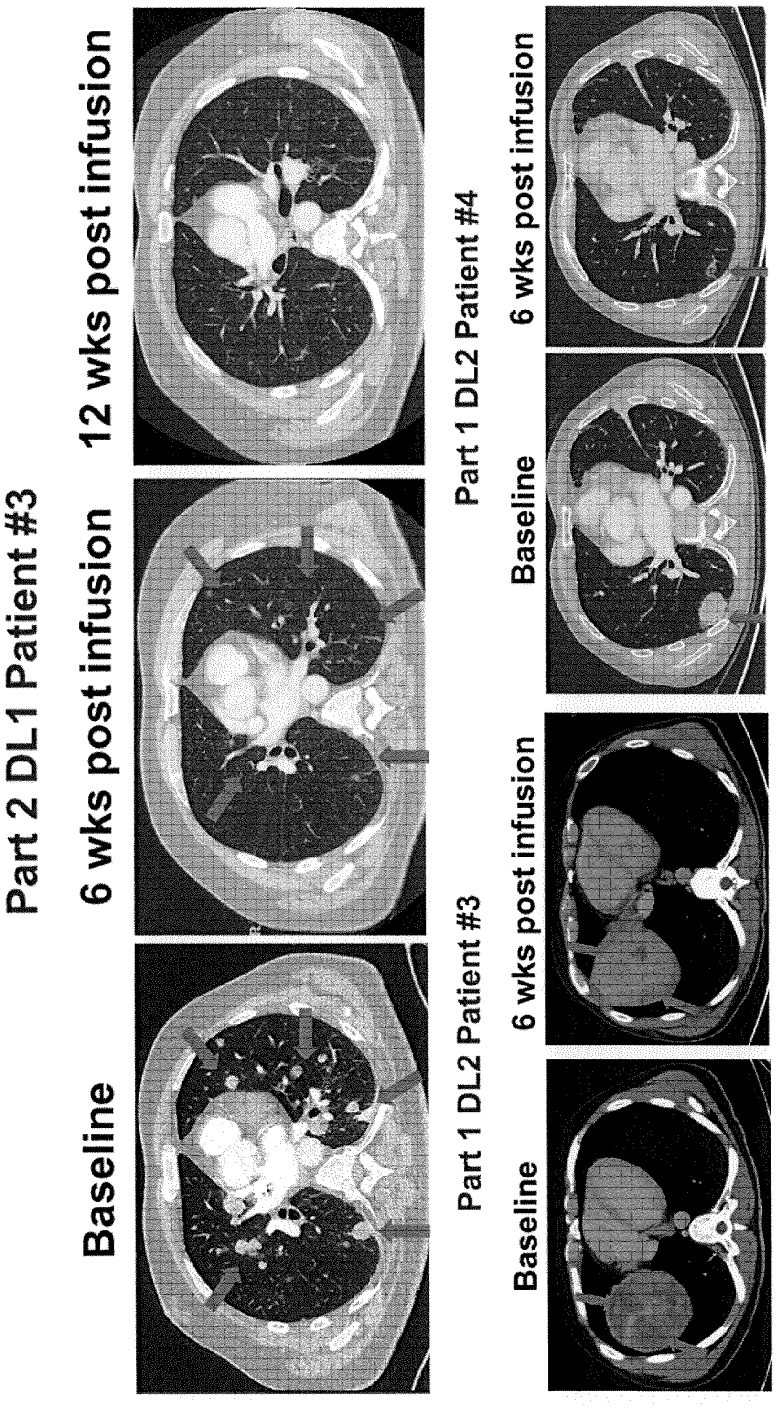

First tumor assessments indicate clinical activity:
4 PR, 4 SD+, 1 SD at 6 weeks post infusion (ORR 4/10, DCR 9/10)
CR
PR
SD+
SD
PD
Redosing
EoT
Part1 DL1
Part2 DL1
Part1 DL2
Part2 DL2
Pat#1
Pat#2
Pat#3
Pat#4
Pat#5
Pat#6
Pat#1*
ACT
6wks
12wks
18wks
24wks
30wks
36wks
42wks
48wks

Figure 15


Part 2 DL1 Patient #3
Baseline
6 wks post infusion
12 wks post infusion
Part 1 DL2 Patient #3
Baseline
6 wks post infusion
Part 1 DL2 Patient #4
Baseline
6 wks post infusion

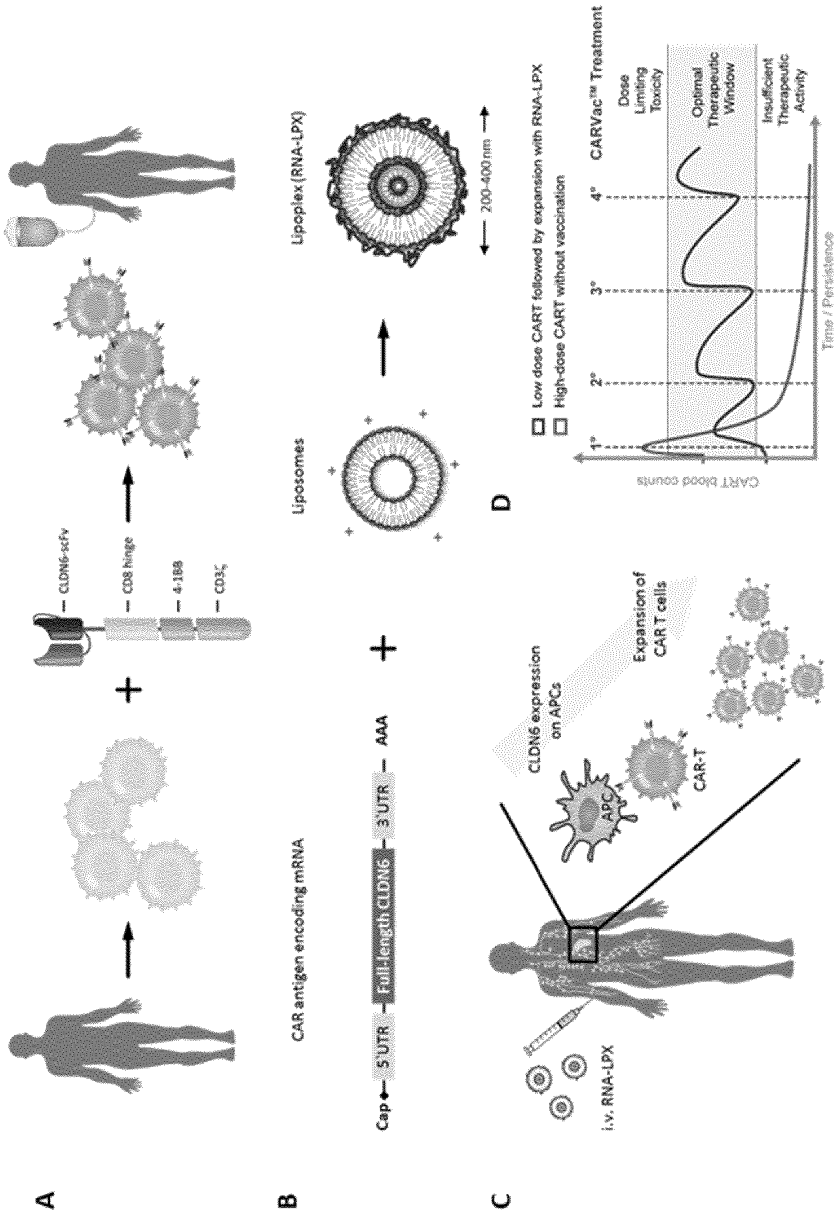

A
CLDN6-scFv
CD8 hinge
4-1BB
CD3ζ
B
CAR antigen encoding mRNA
Cap
5'UTR
Full-length CLDN6
3'UTR
AAA
Liposomes
Lipoplex (RNA-LPX)
200-400nm
C
i.v. RNA-LPX
CLDN6 expression on APCs
APC
CAR-T
Expansion of CAR T cells
D
Low dose CART followed by expansion with RNA-LPX
High-dose CART without vaccination
CARVac™ Treatment
Dose Limiting Toxicity
Optimal Therapeutic Window
Insufficient Therapeutic Activity
1°
2°
3°
4°
CART blood counts
Time / Persistence

Figure 16

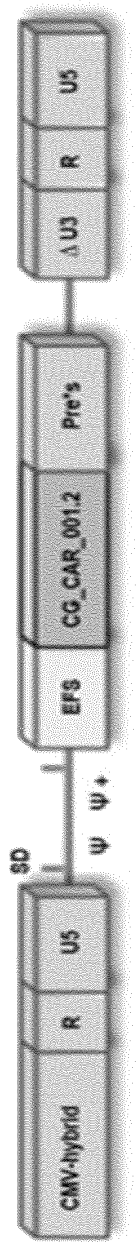


Figure 17

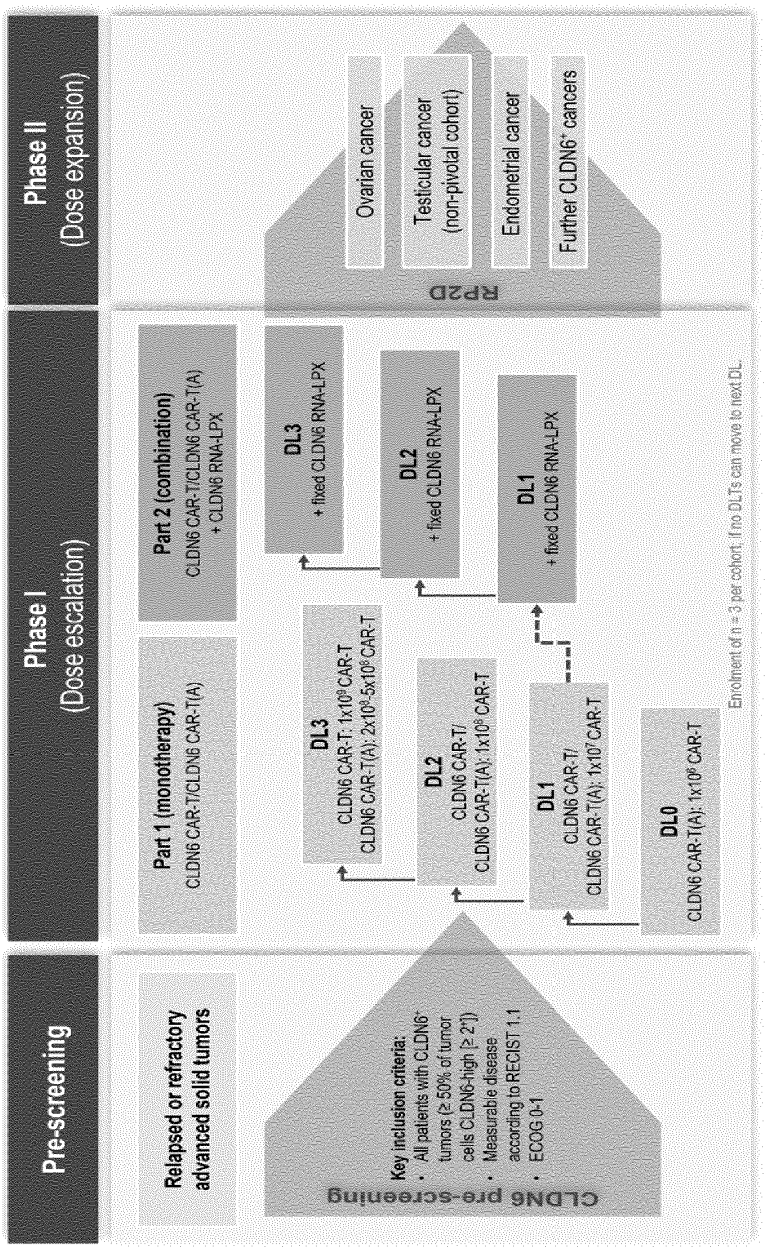

Pre-screening
Phase I
(Dose escalation)
Phase II
(Dose expansion)
Relapsed or refractory advanced solid tumors
CLDN6 pre-screening
Key inclusion criteria:
• All patients with CLDN6+ tumors (≥ 50% of tumor cells CLDN6-high (≥ 2+))
• Measurable disease according to RECIST 1.1
• ECOG 0-1
Part 1 (monotherapy)
CLDN6 CAR-T/CLDN6 CAR-T(A)
Part 2 (combination)
CLDN6 CAR-T/CLDN6 CAR-T(A) + CLDN6 RNA-LPX
DL0
CLDN6 CAR-T(A): 1x10⁸ CAR-T
DL1
CLDN6 CAR-T/ CLDN6 CAR-T(A): 1x10⁷ CAR-T
DL2
CLDN6 CAR-T/ CLDN6 CAR-T(A): 1x10⁸ CAR-T
DL3
CLDN6 CAR-T: 1x10⁸ CAR-T CLDN6 CAR-T(A): 2x10⁸-5x10⁸ CAR-T
DL1
+ fixed CLDN6 RNA-LPX
DL2
+ fixed CLDN6 RNA-LPX
DL3
+ fixed CLDN6 RNA-LPX
Enrolment of n = 3 per cohort, if no DLTs can move to next DL
RP2D
Ovarian cancer
Testicular cancer (non-pivotal cohort)
Endometrial cancer
Further CLDN6+ cancers

Figure 18

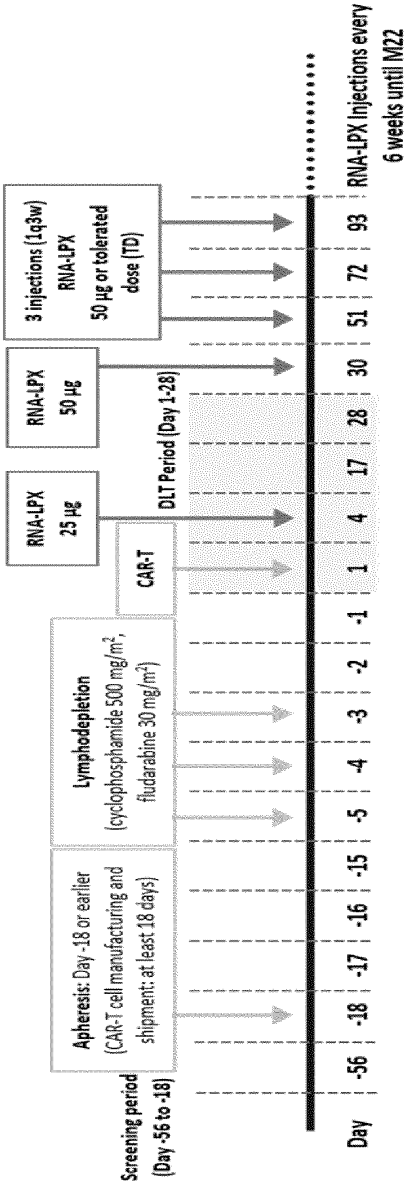

Screening period (Day -56 to -18)
Apheresis: Day -18 or earlier (CAR-T cell manufacturing and shipment: at least 18 days)
Lymphodepletion (cyclophosphamide 500 mg/m², fludarabine 30 mg/m²)
CAR-T
RNA-LPX 25 µg
RNA-LPX 50 µg
3 injections (1q3w) RNA-LPX 50 µg or tolerated dose (TD)
DLT Period (Day 1-28)
RNA-LPX Injections every 6 weeks until M22
Day
-56
-18
-17
-16
-15
-5
-4
-3
-2
-1
1
4
17
28
30
51
72
93

Figure 19

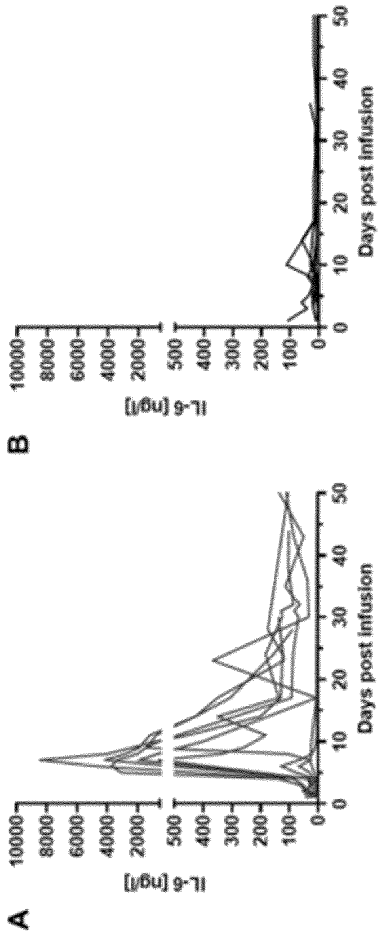

A
B
IL-6 [ng/l]
10000
8000
6000
4000
2000
500
400
300
200
100
0
0
10
20
30
40
50
Days post infusion

Figure 20

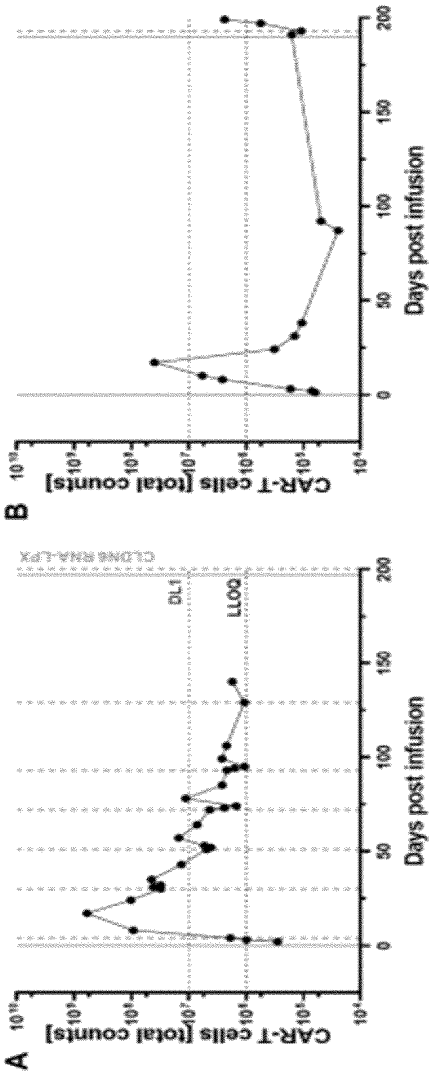

A
B
CAR-T cells [total counts]
Days post infusion
DL1
LLOQ
CLONE RNA-LPX

Figure 21

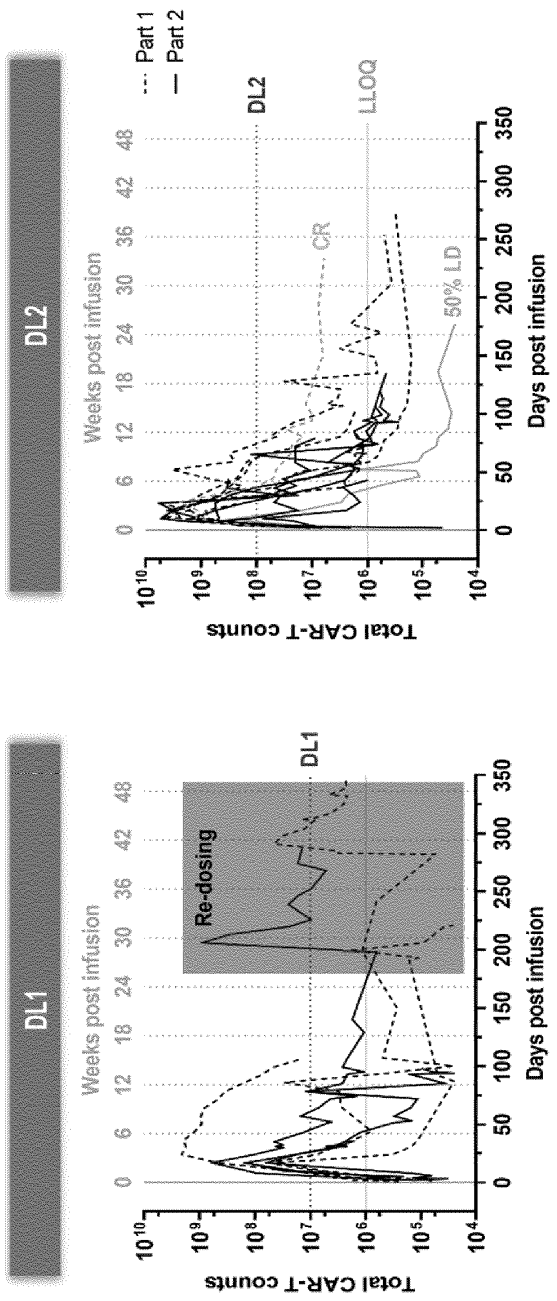

DL2
Part 1
Part 2
DL2
LLOQ
CR
50% LD
Weeks post infusion
Days post infusion
Total CAR-T counts
DL1
Re-dosing
DL1
Weeks post infusion
Days post infusion
Total CAR-T counts

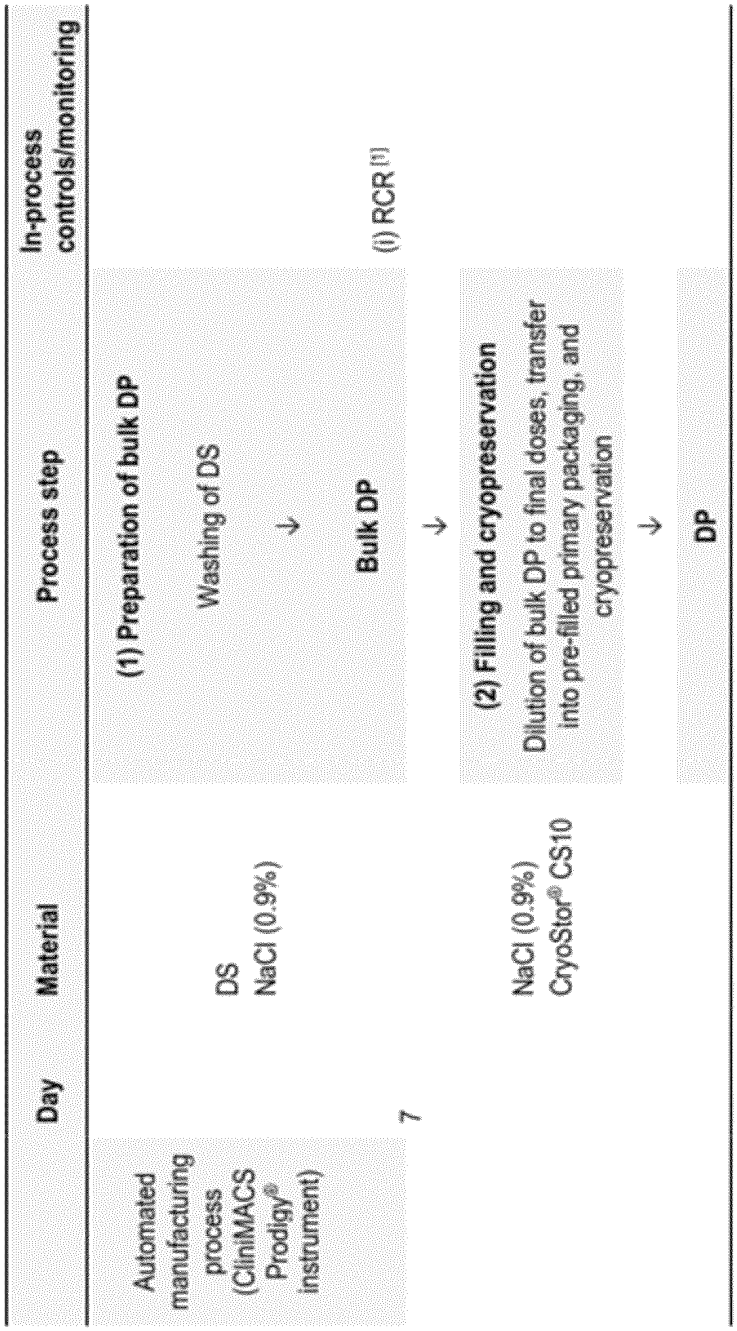
Figure 22

Figure 23

Automated manufacturing process (Prodigy® system)

Day	Material	Process steps	In-process controls/Monitoring
0	**Apheresis material** CD4 and CD8 GMP MicroBeads	**(1) Separation of CD4⁺/CD8⁺ cells** Enrichment of T cells by CD4 and CD8 purification	(i) Cell concentration (ii) Viability (iii) Immune cell composition (iv) Microbial control
↓			
0-2	CD3/CD28 TransAct™ Culture media IL-7IL-15	**(2) Stimulation of CD4⁺ and CD8⁺ T cells** (37°C; 5% CO_2) Cultivation and stimulation of T cells in centricult unit of Prodigy®	(i) Cell concentration (ii) Viability (iii) Immune cell composition
↓			
2	**Viral vector containing supernatant** Vectofusin®-1 X-VIVO™ 15 media IL-7 IL-15	**(3) Transduction of CD4⁺ and CD8⁺ T cells** (MOI 2, 400 x g, 2 h at 32°C) Spinoculation in Prodigy® centricult unit	(i) Cell concentration and viability (ii) Microbial control
↓			
3	Culture media IL-7 IL-15	**(4) Expansion of cells** Washing of CD4⁺ and CD8⁺ T cells, addition of 200 mL culture medium with cytokines and culture of cells in culture unit (37°C; 5% CO_2)	
5	Culture media IL-7 IL-15	Media exchange: Centrifugation of cells, removal of 130 mL medium and addition of 180 mL culture medium with cytokines	
↓			
7		**(5) DS**	

Figure 24


Day
Material
Process step
In-process controls/monitoring
Automated manufacturing process (CliniMACS Prodigy® instrument)
7
(1) Preparation of bulk DP
DS
NaCl (0.9%)
Washing of DS
Bulk DP
(i) RCR [1]
(2) Filling and cryopreservation
NaCl (0.9%)
CryoStor® CS10
Dilution of bulk DP to final doses, transfer into pre-filled primary packaging, and cryopreservation
DP