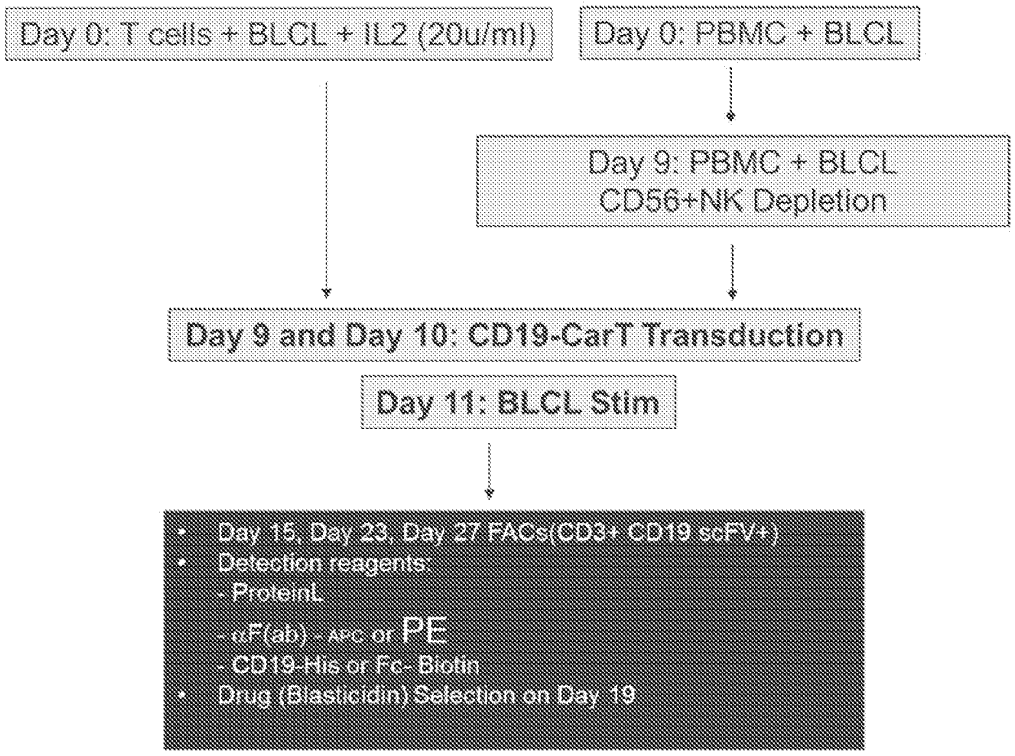
Figure 1


Day 0: T cells + BLCL + IL2 (20u/ml)
Day 0: PBMC + BLCL
Day 9: PBMC + BLCL
CD56+NK Depletion
Day 9 and Day 10: CD19-CarT Transduction
Day 11: BLCL Stim
• Day 15, Day 23, Day 27 FACs(CD3+ CD19 scFV+)
• Detection reagents:
- ProteinL
- αF(ab) - APC or PE
- CD19 His or Fc- Biotin
• Drug (Blasticidin) Selection on Day 19

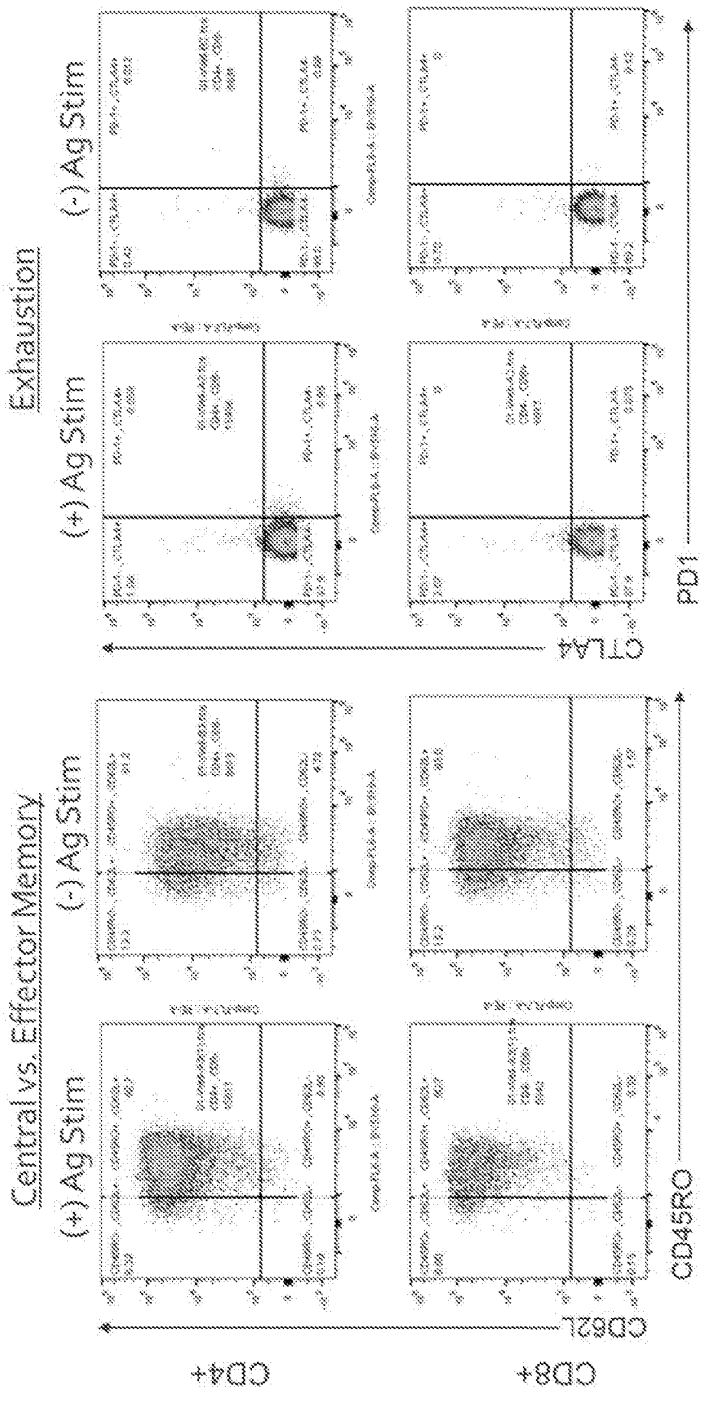
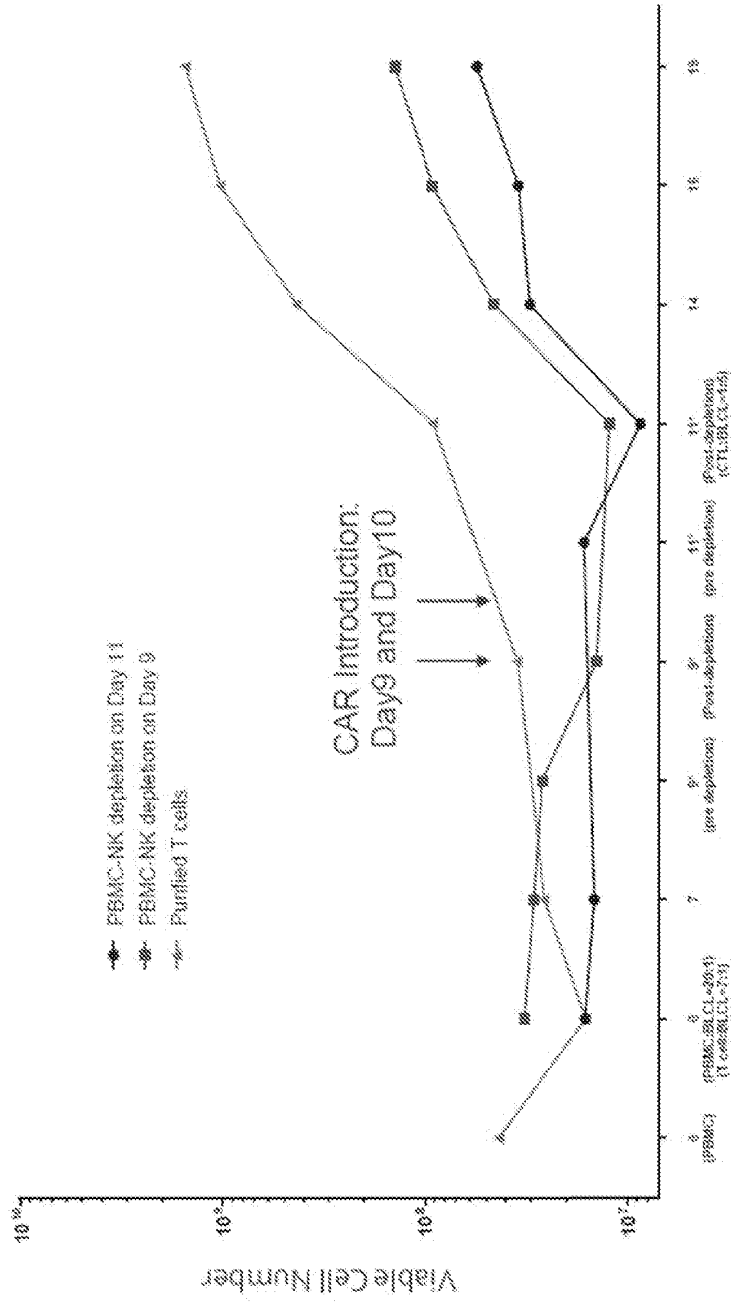

Central vs. Effector Memory
Exhaustion
(+) Ag Stim
(-) Ag Stim
(+) Ag Stim
(-) Ag Stim
CD4+
CD8+
CD62L
CD45RO
CTLA4
PD1

Figure 2

Figure 3


Growth Curve of T cells Stimulated by Autologous BLCL
PBMC-NK depletion on Day 11
PBMC-NK depletion on Day 9
Purified T cells
CAR Introduction:
Day9 and Day10
Viable Cell Number
Day / Process

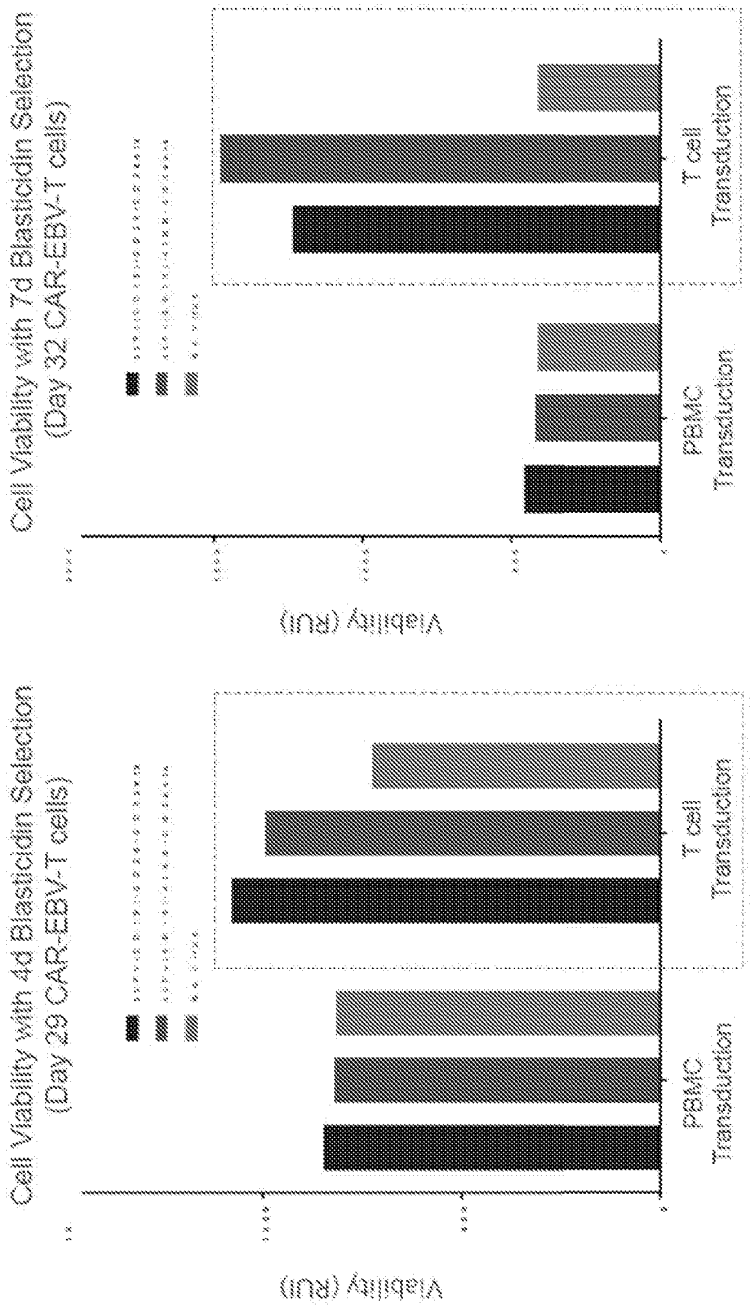
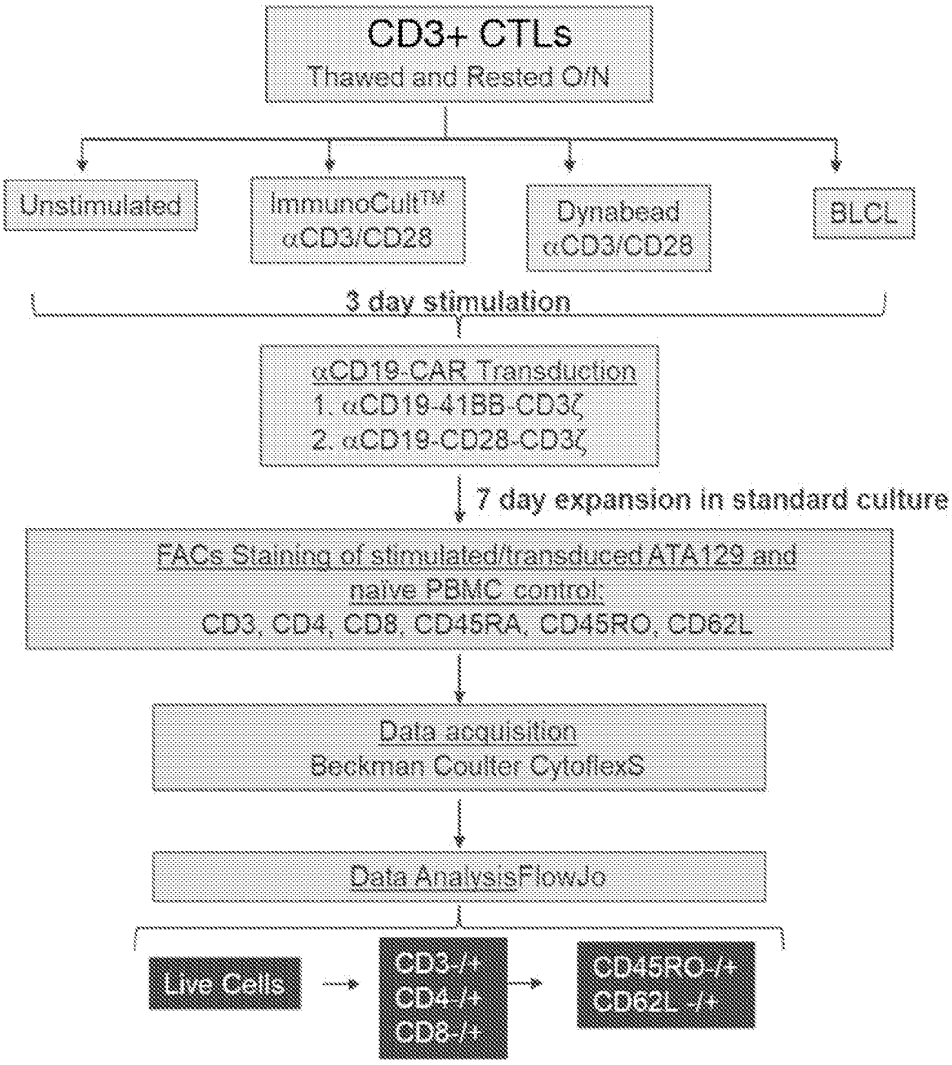

Cell Viability with 7d Blasticidin Selection
(Day 32 CAR-EBV-T cells)
Viability (RUI)
PBMC Transduction
T cell Transduction
Cell Viability with 4d Blasticidin Selection
(Day 29 CAR-EBV-T cells)
Viability (RUI)
PBMC Transduction
T cell Transduction

Figure 4

Figure 5


CD3+ CTLs
Thawed and Rested O/N
Unstimulated
ImmunoCult™ αCD3/CD28
Dynabead αCD3/CD28
BLCL
3 day stimulation
αCD19-CAR Transduction
1. αCD19-41BB-CD3ζ
2. αCD19-CD28-CD3ζ
7 day expansion in standard culture
FACs Staining of stimulated/transduced ATA129 and naïve PBMC control:
CD3, CD4, CD8, CD45RA, CD45RO, CD62L
Data acquisition
Beckman Coulter CytoflexS
Data AnalysisFlowJo
Live Cells
CD3-/+
CD4-/+
CD8-/+
CD45RO-/+
CD62L-/+

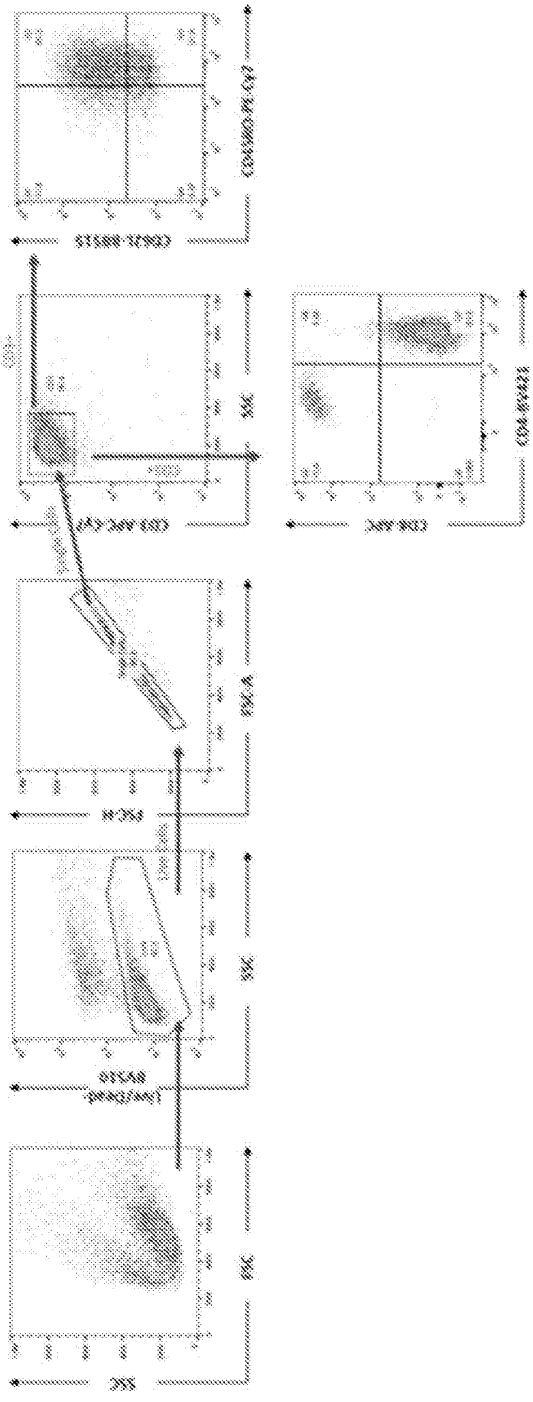
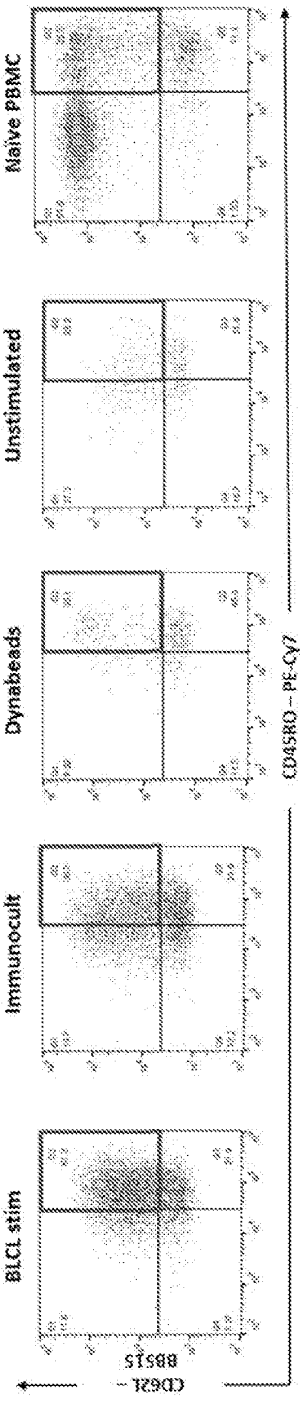


Figure 6

Figure 7


Naïve PBMC
Unstimulated
Dynabeads
Immunocult
BLCL stim
CD45RO – PE-Cy7
CD62L – BB515

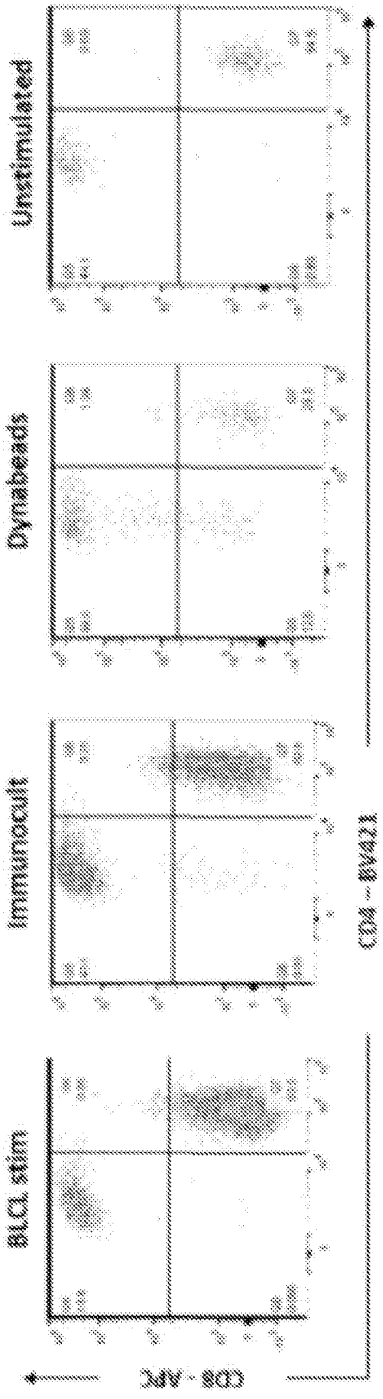
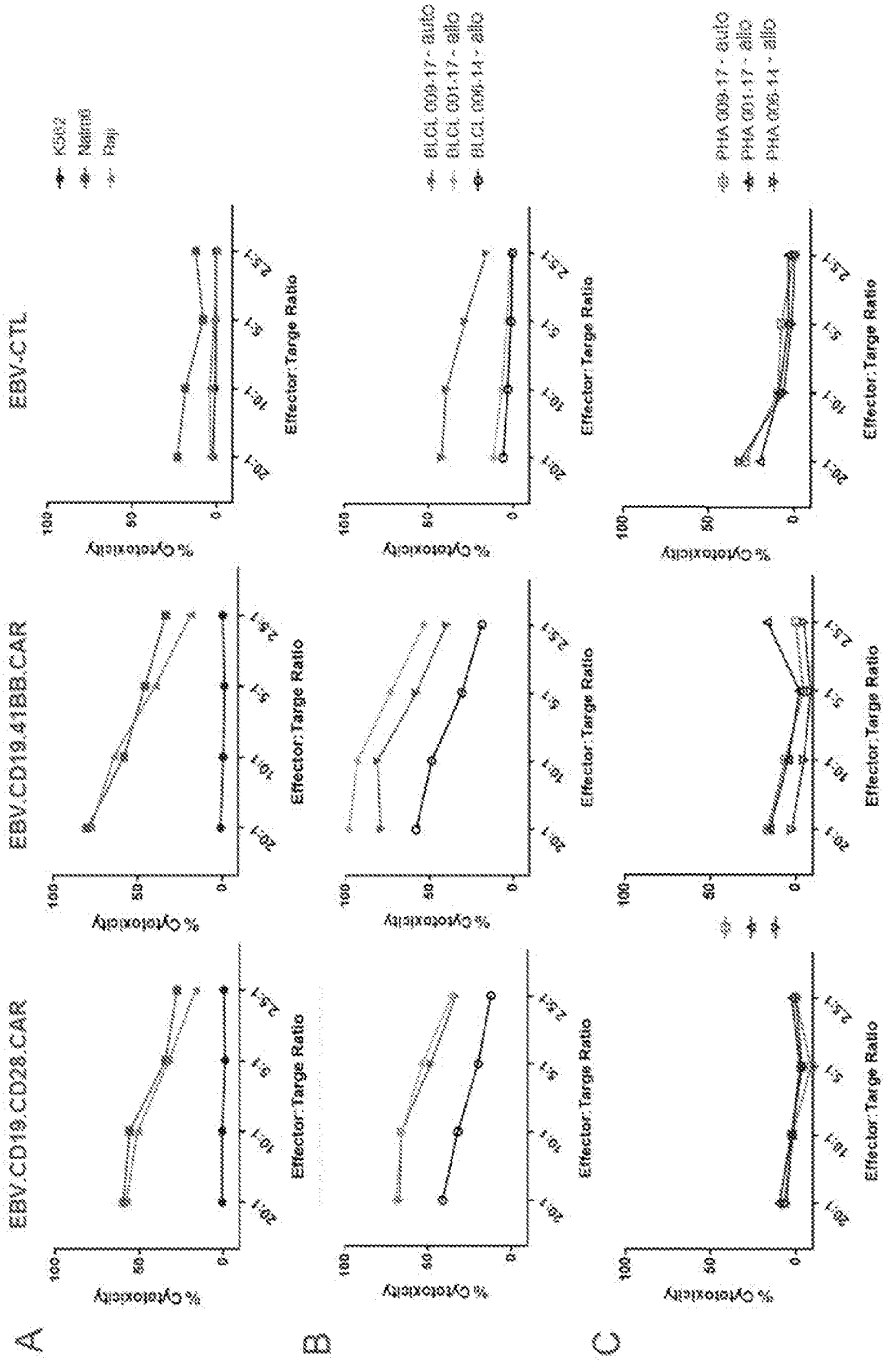

Unstimulated
Dynabeads
Immunocult
BLCA stim
CD8 - APC
CD4 - BV421

Figure 8

Figure 9


A
B
C
EBV.CD19.CD28.CAR
EBV.CD19.41BB.CAR
EBV-CTL
% Cytotoxicity
Effector:Targe Ratio
K562
Raji
BLCL 009-17 - auto
BLCL 001-17 - allo
BLCL 006-14 - allo
PHA 009-17 - auto
PHA 001-17 - allo
PHA 006-14 - allo

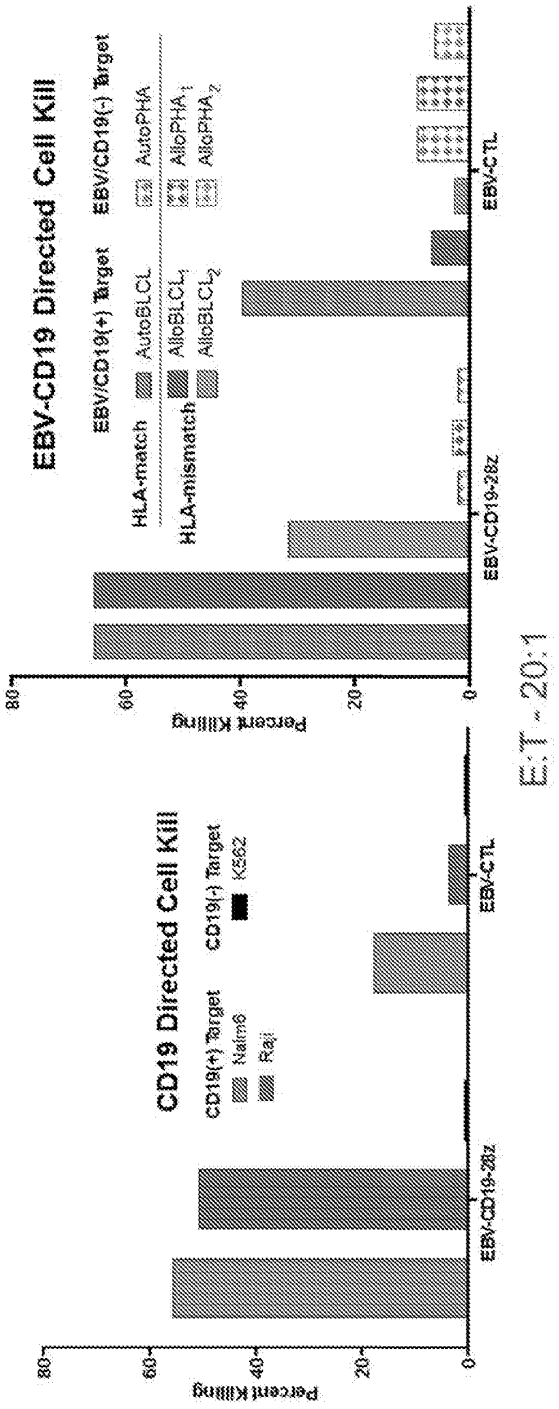

EBV-CD19 Directed Cell Kill
EBV/CD19(+) Target
EBV/CD19(-) Target
HLA-match
AutoBLCL
AutoPHA
HLA-mismatch
AlloBLCL1
AlloBLCL2
AlloPHA1
AlloPHA2
Percent Killing
80
60
40
20
0
EBV-CD19-28z
EBV-CTL
CD19 Directed Cell Kill
CD19(+) Target
Nalm6
Raji
CD19(-) Target
K562
E:T ~ 20:1

Figure 10

Figure 11

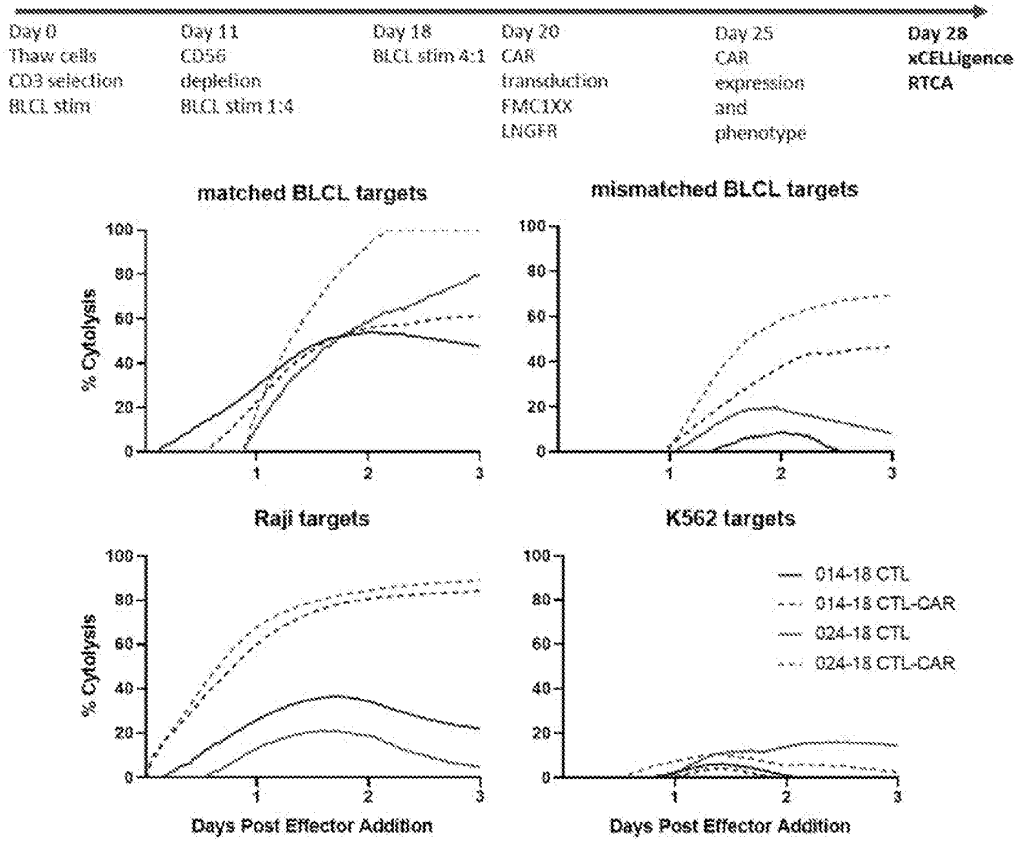

Day 0
Thaw cells
CD3 selection
BLCL stim
Day 11
CD56
depletion
BLCL stim 1:4
Day 18
BLCL stim 4:1
Day 20
CAR
transduction
FMCIXX
LNGFR
Day 25
CAR
expression
and
phenotype
Day 28
xCELLigence
RTCA
matched BLCL targets
mismatched BLCL targets
Raji targets
K562 targets
% Cytolysis
Days Post Effector Addition
014-18 CTL
014-18 CTL-CAR
024-18 CTL
024-18 CTL-CAR

Figure 12

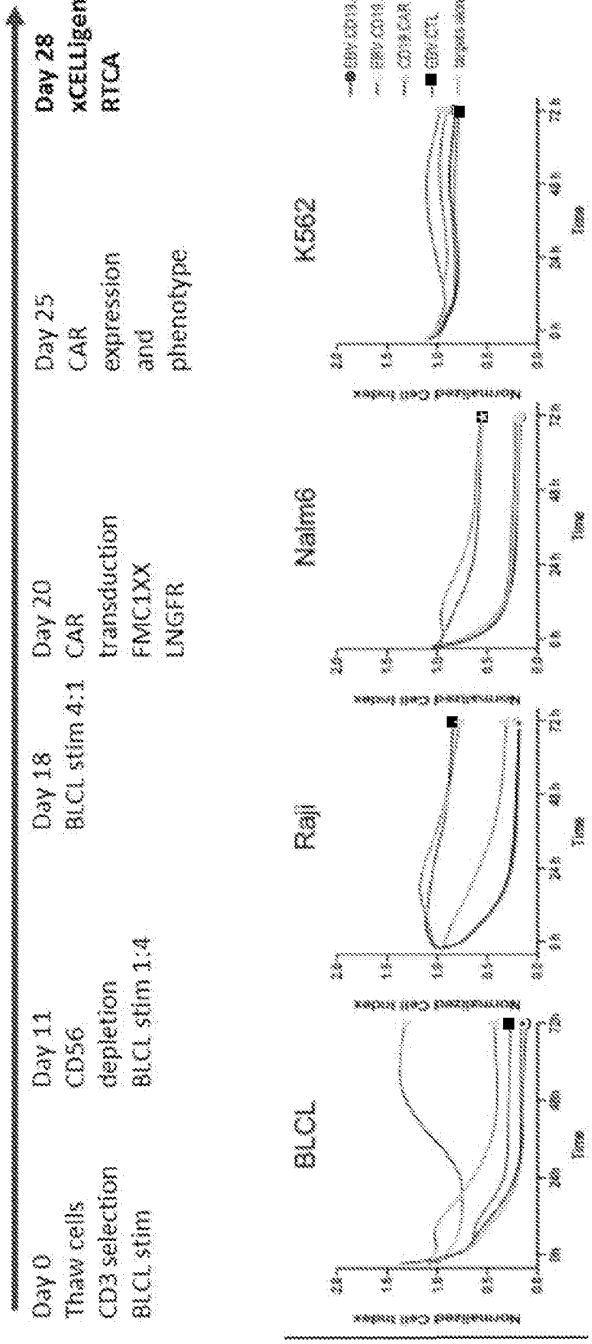
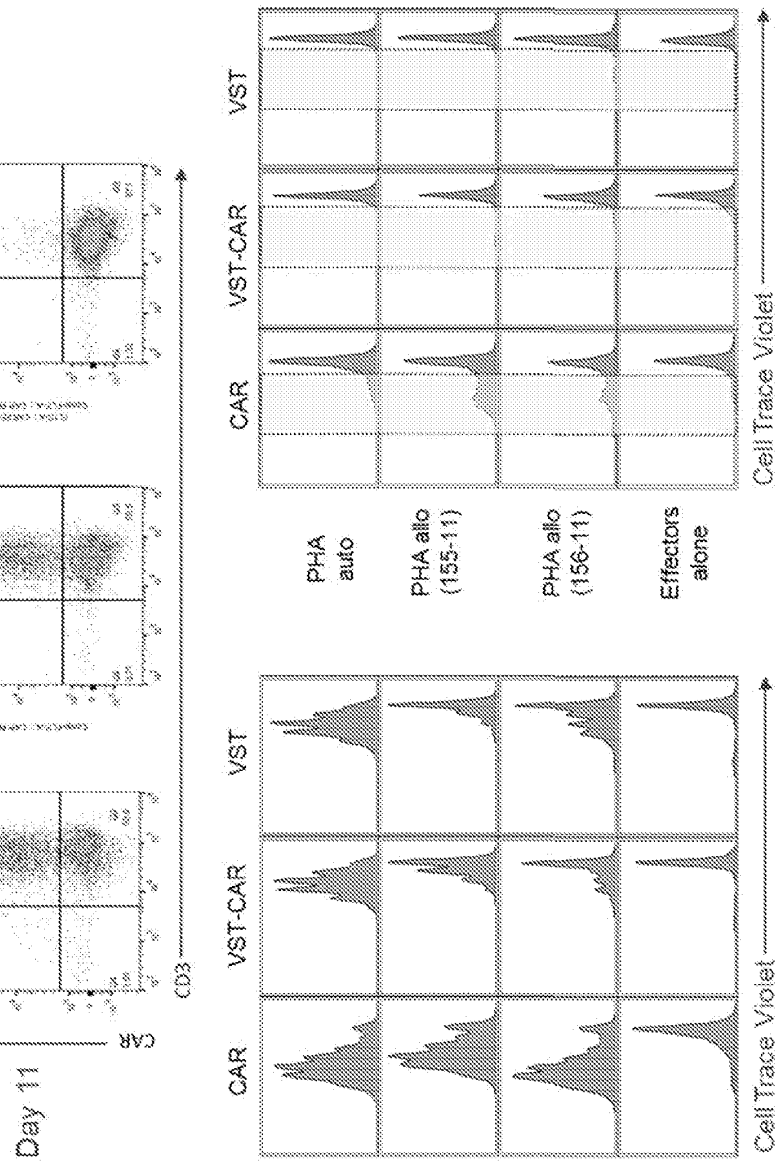

Day 0
Thaw cells
CD3 selection
BLCL stim
Day 11
CD56
depletion
BLCL stim 1:4
Day 18
BLCL stim 4:1
Day 20
CAR
transduction
FMC1XX
LNGFR
Day 25
CAR
expression
and
phenotype
Day 28
xCELLigence
RTCA
BLCL
Raji
Nalm6
K562
Time

Figure 13


FMC63 CAR
VST-FMC63 CAR
VST
47.8%
32.8%
Day 11
CD3
CAR
VST-CAR
VST
BLCL
Raji
NALM6
K562
Cell Trace Violet
PHA auto
PHA allo (155-11)
PHA allo (156-11)
Effectors alone

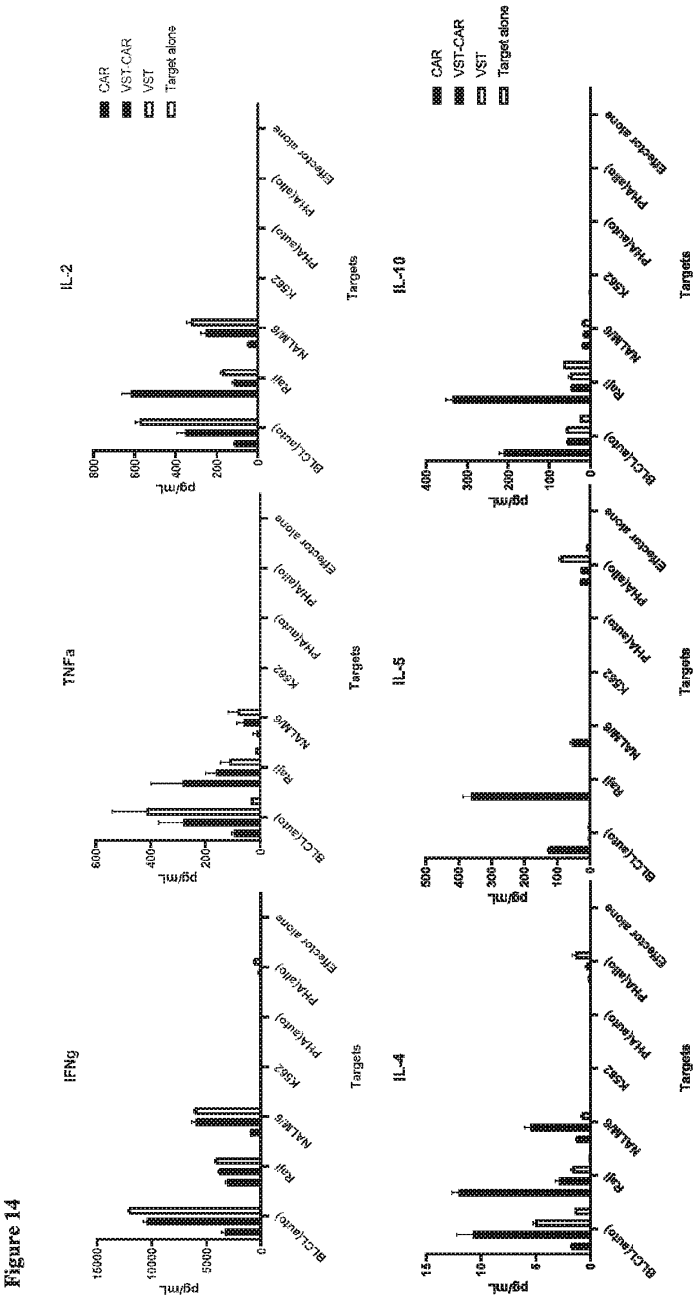

IFNg
TNFa
IL-2
IL-4
IL-5
IL-10
CAR
VST-CAR
VST
Target alone
pg/mL
Targets
BLCL(auto)
Raji
NALM6
K562
PHA(auto)
PHA(allo)
Effector alone

Figure 14