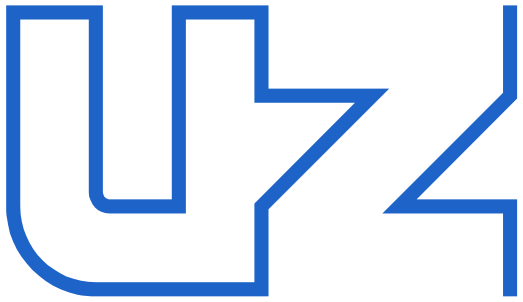


UZ
GENT

UNIVERSITEIT
GENT

CAR-T and solid cancers : hope or hype ?

Sylvie Rottey MD, PhD

Medical Oncology
Drug Research Unit Gent

November 24 2018

UZ GENT

UNIVERSITEIT GENT

Genetic redirection of T lymphocytes with CARS (chimeric antigen receptors)

To date solid tumors are less susceptible to CAR therapies and instead have been treated more successfully with immune checkpoint blockade

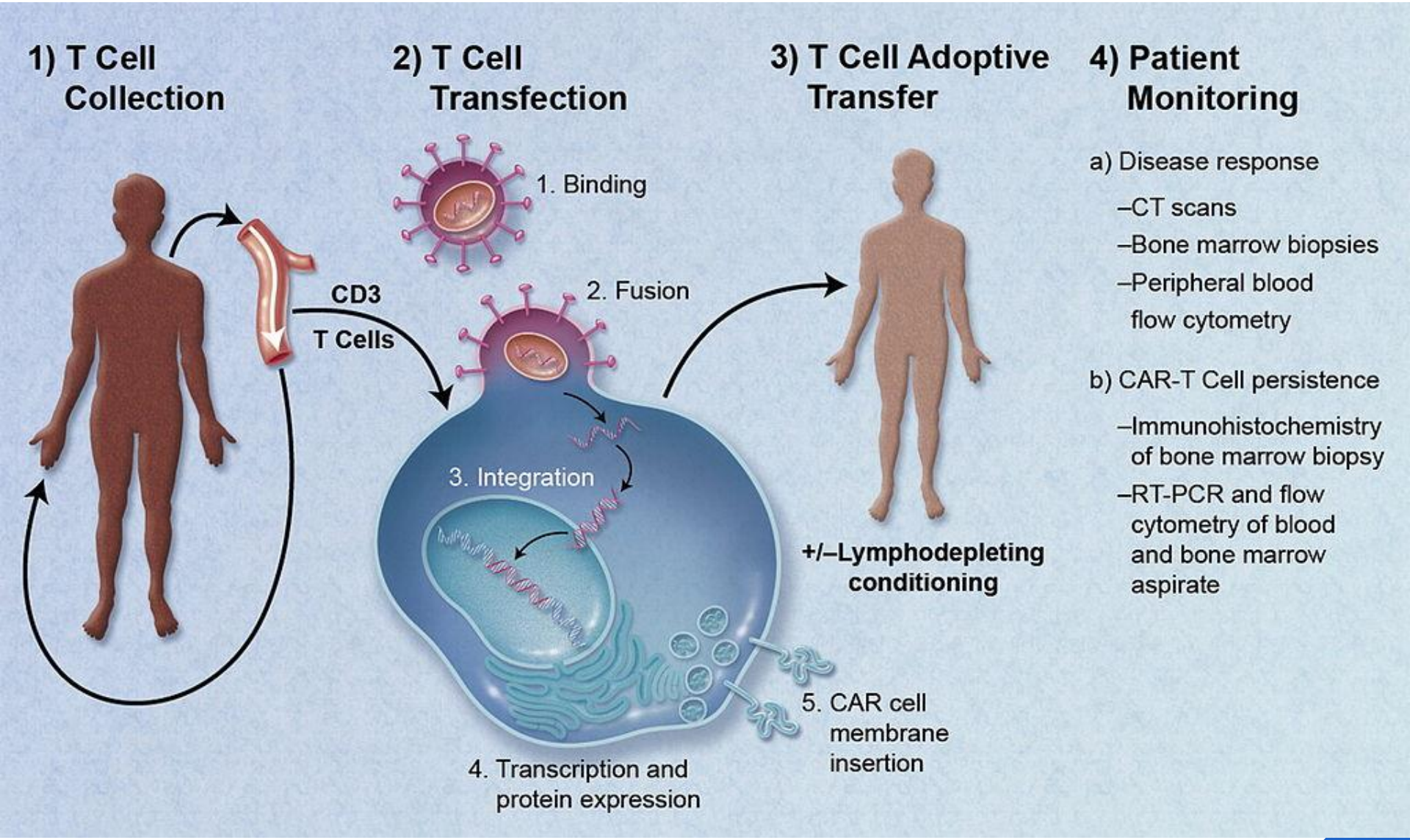
ACT : adoptive cell transfer

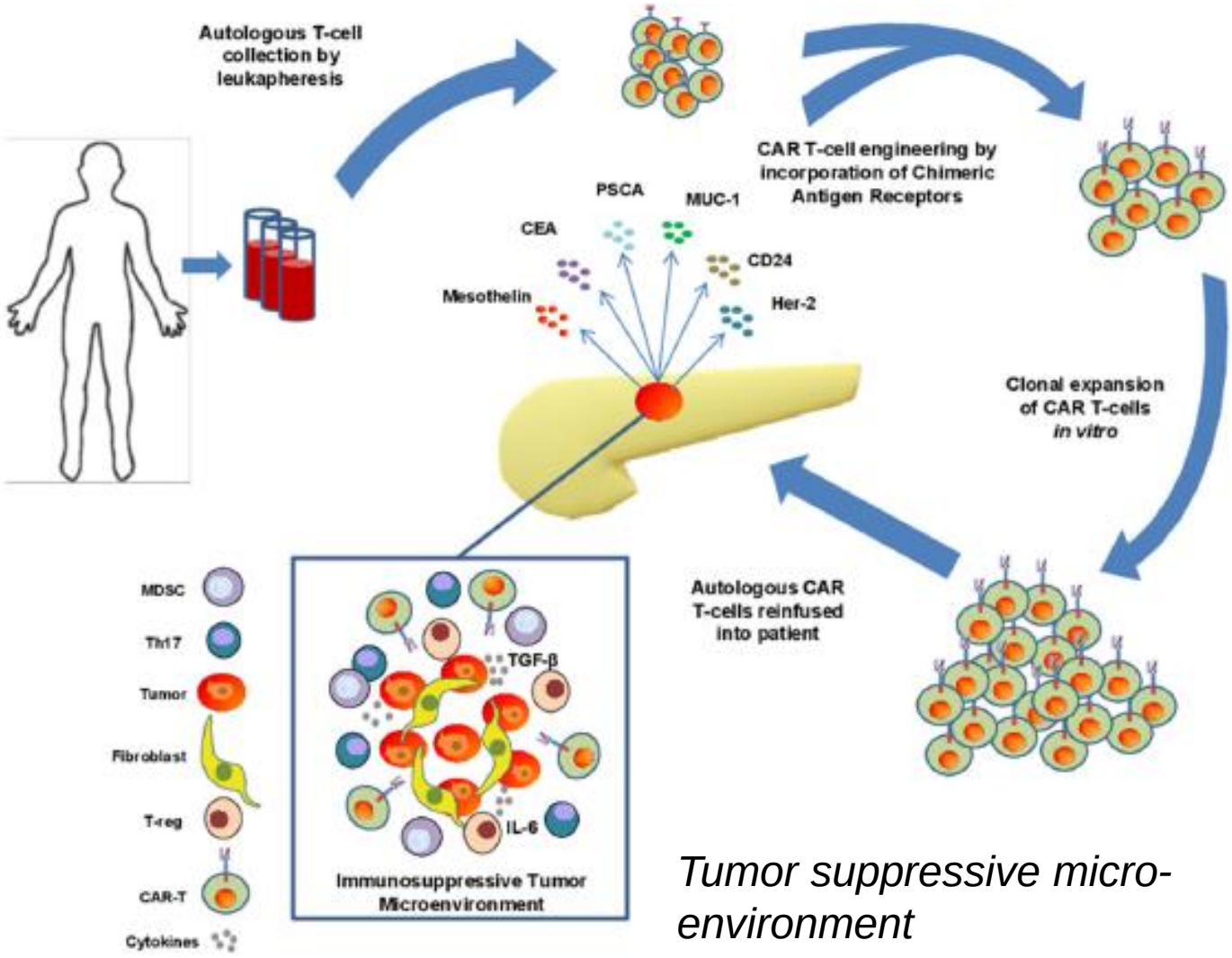
***Ex vivo enrichment of tumor specific cells**

***Expansion to large numbers**

***Reinfusion into the patient**

non-therapeutic endogenous lymphocytes obtained from the peripheral blood can be rendered tumor specific via genetic redirection with a T-cell receptor or chimeric antigen receptor (CAR)

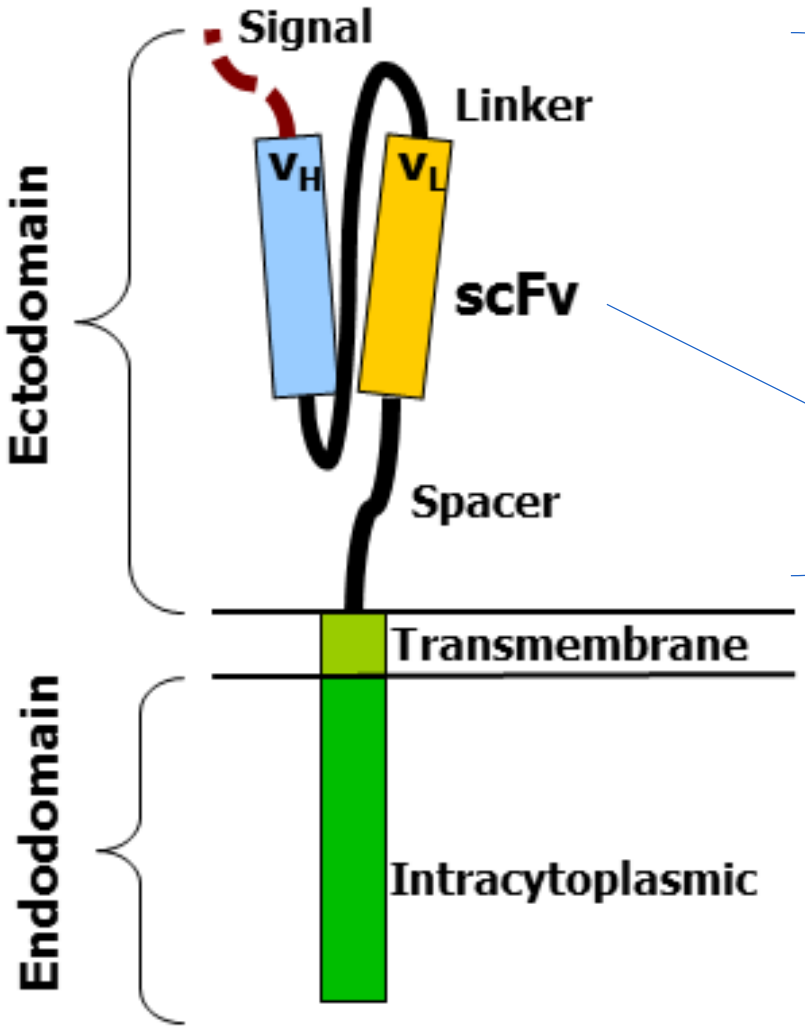

1) T Cell Collection
2) T Cell Transfection
3) T Cell Adoptive Transfer
4) Patient Monitoring
CD3
T Cells
1. Binding
2. Fusion
3. Integration
4. Transcription and protein expression
5. CAR cell membrane insertion
+/–Lymphodepleting conditioning
a) Disease response
–CT scans
–Bone marrow biopsies
–Peripheral blood flow cytometry
b) CAR-T Cell persistence
–Immunohistochemistry of bone marrow biopsy
–RT-PCR and flow cytometry of blood and bone marrow aspirate



Tumor suppressive micro-environment

FIGURE 1 | Isolation, engineering, and challenges of CAR T cell therapy in pancreatic adenocarcinoma (PDAC). T cells are collected from peripheral blood of patients with PDAC via leukapheresis and engineered to express chimeric antigen receptors directed toward a specific tumor antigen. These cells are subsequently expanded before reinfusion into patients. Significant challenges exist for these cells to infiltrate the immunosuppressive tumor microenvironement of PDAC including the presence of dense stroma and myofibroblast cells, immunosuppressive cytokines such as IL-6 and TGF-β, and the presence of immunosuppressive immune cell types such as Th17 cells, MDSCs, and suppressive T-regs.

CAR = chimeric antigen receptor



Peptide recognition region

Single chain variable fragment

the functional end of the receptor- after antigen recognition, receptors cluster and a signal is transmitted to the cell

CAR –redirected T cell therapies

Have been successful in hematologic malignancies

CAR T cells directed to CD19

ALL

DLBCL

CLL

other B cell non-Hodgkin Lymphomas

FDA approval Tisagenlecleucel (B-ALL and DLBCL)
Axicabtagen ciloleucel (DLBCL)

CAVE TOXICITIES : cytokine release and neuropathy

CAR T cells in solid tumors

CAVE severe toxicities since the targeted antigens are often not completely foreign to the host

Off tumor 'response'

*RCC : carbonic anhydrase IX (CAIX) liver toxicity (expression bile duct)

*CRC patient : ERBB2 : high dosage : multiorgan failure with lung toxicity (AG expression lung epithelium)

*GI tumors CEACAM-5-CAR T cells : poor efficacy and persistence of cells + toxicity from expression on lung epithelium

CAREFUL CONSIDERATION OF TARGET ANTIGENS

CAR T cells in solid tumors

More specific antigens :
more limited off-tumor effects but poor clinical efficacy

*HER2-based CAR in sarcoma

*mesothelin-specific CAR in mesothelioma and pancreatic ca

*CEA for colorectal ca

*EGFRvIII in glioblastoma

*α-folate receptor in ovarian ca

Safer but only stable diseases as best result

Success : glioblastoma : localized delivery CART cells engineered against IL-13Rα : ORR of 7.5 months !!

CAR T cells in solid tumors

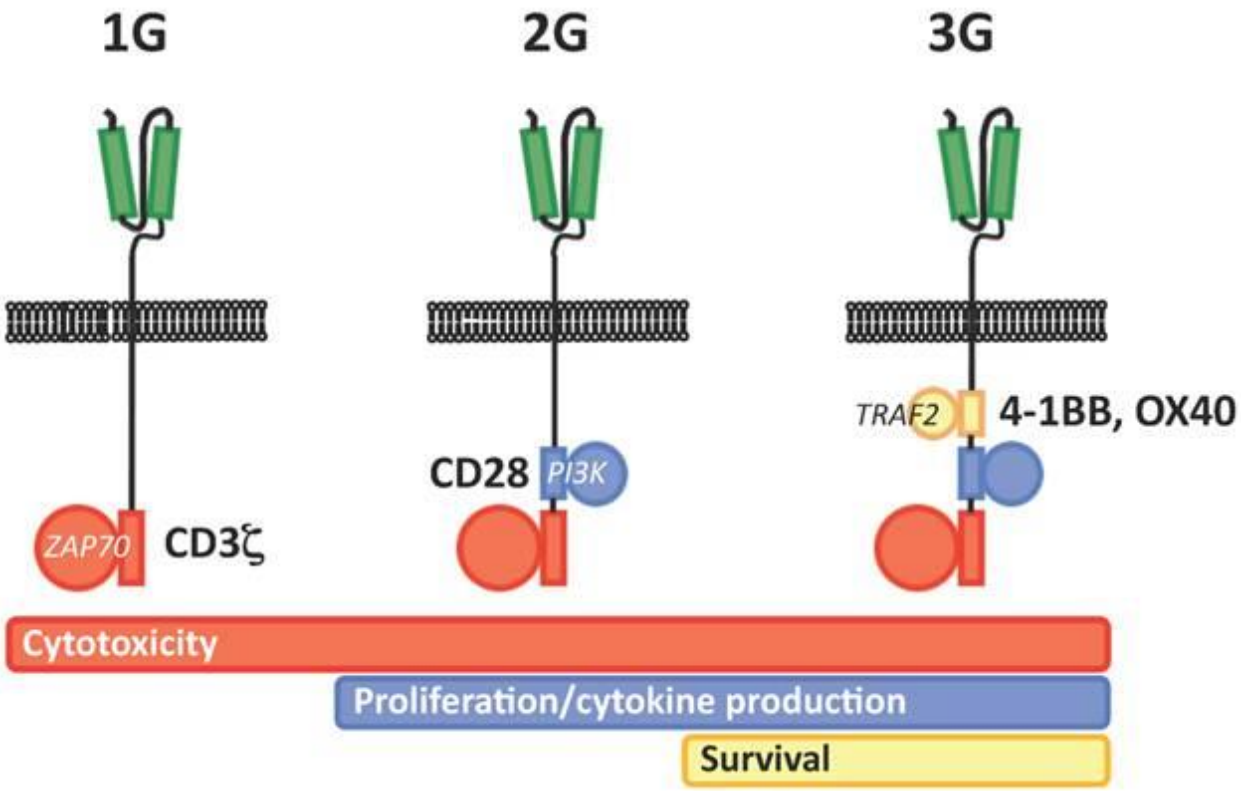
Even if the perfect antigen can be determined

Challenges : solid tumors versus liquid tumors !

-poor trafficking to the tumor site

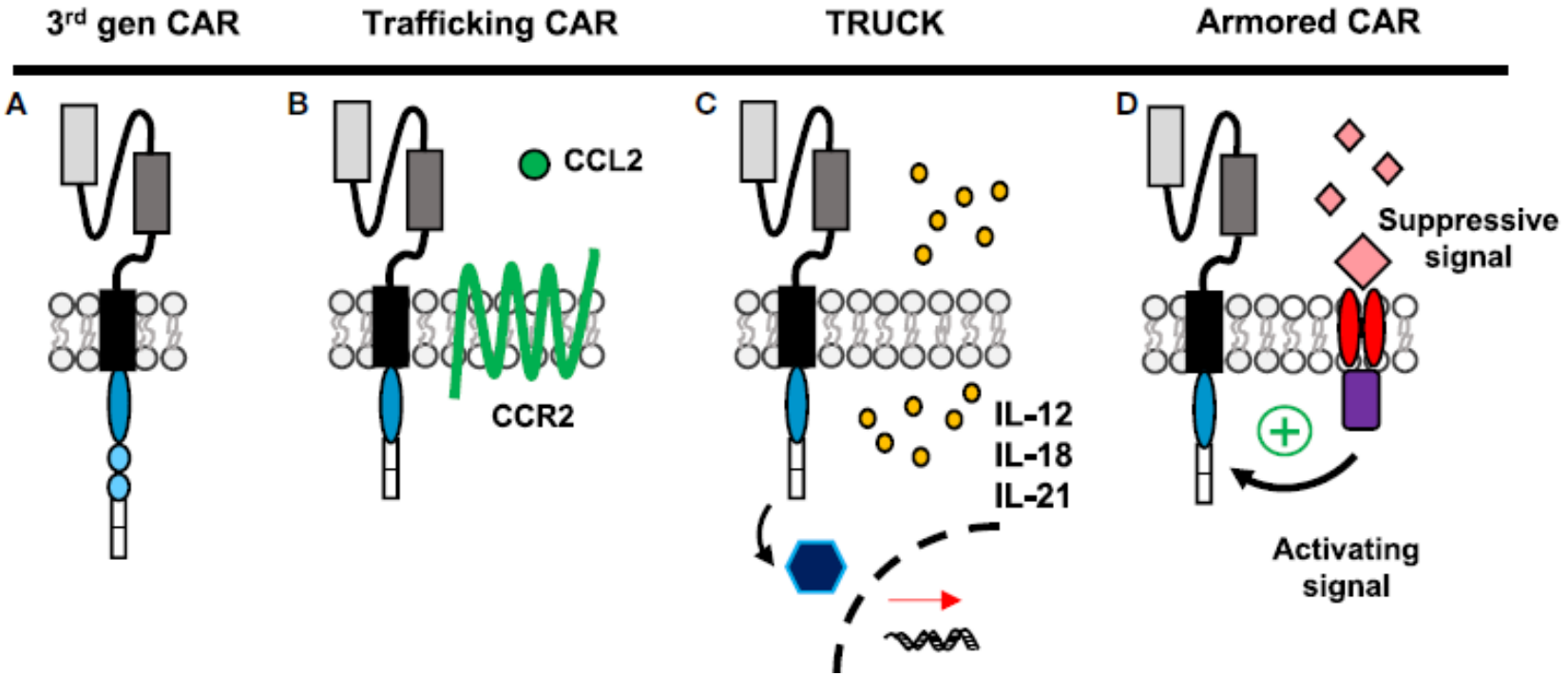
-limited persistence and proliferation within the host

-CAR T cells can be functionally suppressed by the tumor microenvironment



Second generation CARs also contain co-stimulatory domains, like CD28 and/or 4-1BB. The involvement of these intracellular signaling domains improve T cell proliferation, cytokine secretion, resistance to apoptosis, and in vivo persistence

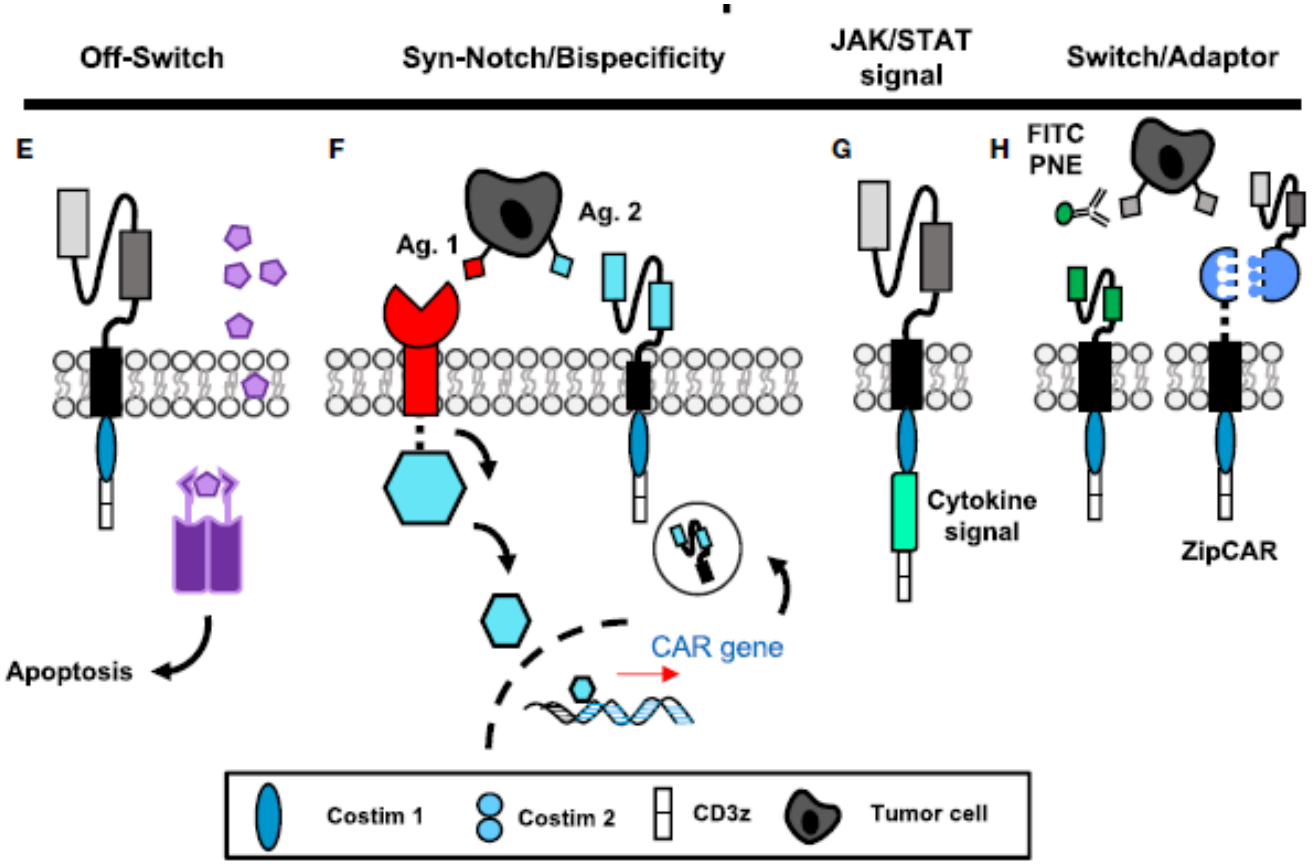
The third-generation CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, to augment T cell activity



Additional chemokine receptors : improved trafficking to tumors

To resist exhaustion

FIGURE 2 | "Fourth-generation" chimeric antigen receptor (CAR) constructs incorporate novel mechanisms to enhance targeted antitumor efficacy. **(A)** The third-generation CAR incorporates the extracellular scFv with intracellular CD3ζ signaling and two tandem costimulatory domains. **(B)** CAR T cells with additional chemokine receptors have improved trafficking to tumors. **(C,G)** T cells secreting additional cytokines or engineered with cytokine signaling domains have enhanced activation and can modulate surrounding microenvironment. **(D)** Armored CARs redirect suppressive signals from the tumor to activating signals to resist exhaustion.



Mitigation of off target therapy

Switchable CAR targeting via adaptor molecules

(E) Suicide genes and **(F)** bispecificity mitigate off-tumor toxicity through the ability to deplete transferred cells or enhance specific targeting to tumors, respectively. **(H)** Switchable CAR targeting *via* adaptor molecules provides versatile opportunity to control CAR activation, specificity, and longevity after transfer of cells. Abbreviations: Ag, antigen; PNE, peptide neo-epitopes.

Future CAR T cell

Knochelman et al. (2018 Frontiers in Immunology)

I. Should be armored with a superior targeting system specific to the tumor and tumor tissue

II. Engineering of a highly potent, persistent, and self renewing T Cell subset is of importance

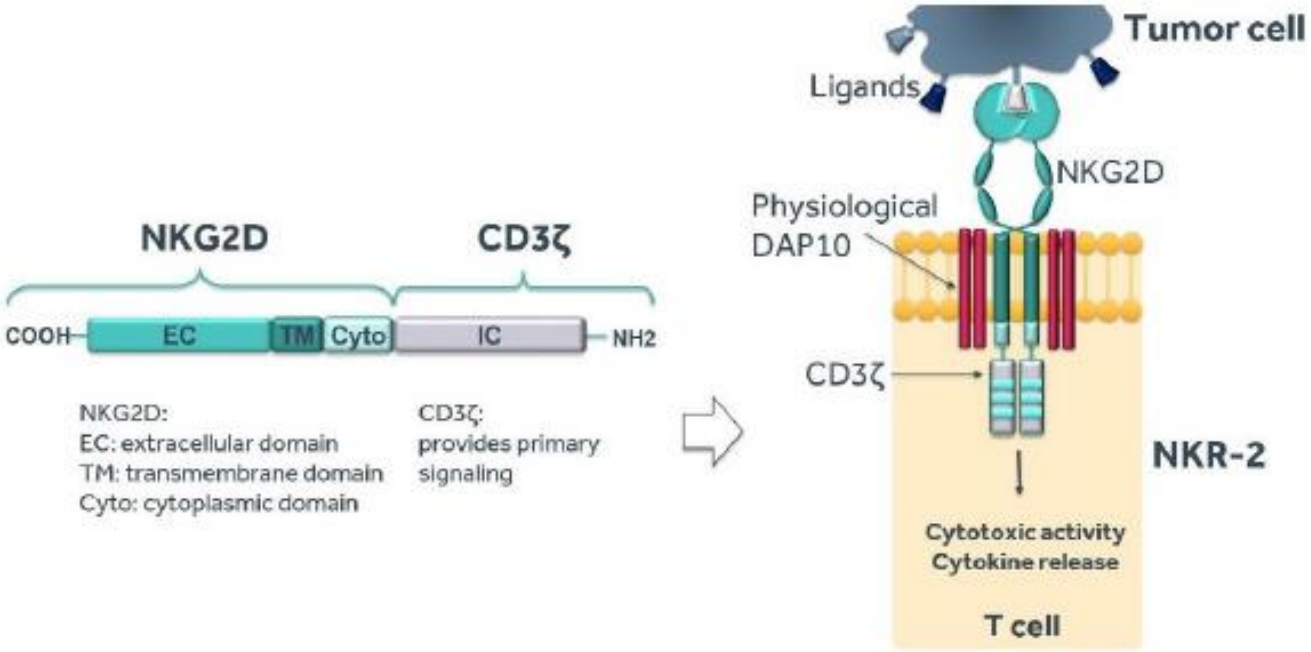
III. Rejuvenation of the endogenous host response through CAR T cell production of monoclonal antibodies against immune checkpoint molecules can bolster the immune attack

TABLE 1 | Clinical trials of fourth-generation chimeric antigen receptor (CAR) T cells in solid tumors.

4th Generation CAR T cells in solid tumors		
ClinicalTrials.gov identification	**Trial description**	**Location(s)**
"Armored" CAR		
NCT03089203	CAR T cells targeting PSMA for castration-resistant prostate cancer with dominant negative TGF-β receptor	University of Pennsy
NCT02937844	Pilot study of autologous chimeric switch receptor modified T cells in recurrent glioblastoma multiforme	Sanbo Brain Hospita University, Beijing, C
NCT02930967	Chimeric switch receptor with PD-L1+ recurrent or metastatic malignant tumors	China Meitan Genera
Suicide genes		
NCT00730613	CAR T against IL-13Ra2 in glioblastoma with Hy/TK suicide switch	City of Hope Medica
NCT02992210	4SCAR-GD2 targeting CAR with iCaspase9 domain in refractory solid tumors	Shenzhen Geno-Imm
NCT02414269	Malignant pleural disease treated with Meso-CAR T cells, modified with iCasp9/M28ζ	Memorial Sloan Kett
NCT01822652	GD-2-CAR T (28-Ox40ζ) and iCaspase9 Suicide safety switch for Neuroblastoma	Baylor College of Me
NCT03185468	4SCAR-GS2 with iCaspase9 domain in advanced/metastatic urothelial carcinoma	Shenzhen Geno-Imm
Antibody-producing CAR T cells		
NCT03179007	CTLA-4/PD-1 antibody expressing MUC-1 CAR T for MUC1+ advanced solid tumors	Shanghai Cell Thera
NCT03182803	CTLA-4/PD-1 antibody expressing mesothelin-CAR T for Meso+ advanced solid tumors	Shanghai Cell Thera
NCT03182816	CTLA-4/PD-1 antibody expressing EGFR-CAR T for EGFR+ advanced solid tumors	Shanghai Cell Thera
NCT02862028	PD-1 antibody expressing CAR T cells for EGFR family member positive advanced solid tumor (liver, lung, stomach)	Shanghai Internation Shanghai, China
NCT02873390	PD-1 antibody expressing CAR T cells for EGFR family member positive advanced solid tumor	Ningbo Cancer Hosp
NCT03030001	PD-1 antibody expressing mesothelin-specific CAR T cells for meso+ malignant tumors (recurrent or refractory)	Ningbo Cancer Hosp
NCT03170141	4SCAR-IgT against EGFRvIII on glioblastoma multiforme, producing PD-1 and PD-L1 antibodies	Shenzhen Geno-Imm

THINK – UZGent

Figure 2: The NKR-2 construct



The NKG2D is a C-type, lectin-like, type II transmembrane glycoprotein receptor expressed on human Natural Killer (NK), NKT, activated CD8+ T-cells and, under some conditions, subset of γδ+ T-cells.

The large majority of colorectal cancer cells (80-100%) express different NKG2D ligands including MIC-A/B, ULBP1-5.

CAR-T and solid cancers : hope or hype ?

Sylvie Rottey MD, PhD

Medical Oncology
Drug Research Unit Gent

November 24 2018

UZ GENT

UNIVERSITEIT GENT