Promises and limitations of nanoparticles in the era of cell therapy: Example with CD19-targeting chimeric antigen receptor (CAR)-modified T cells

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PROMISES AND LIMITATIONS OF NANOPARTICLES IN THE ERA OF CELL THERAPY: EXAMPLE WITH CD19-TARGETING CHIMERIC ANTIGEN RECEPTOR (CAR)-MODIFIED T CELLS

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ABSTRACT

A number of nanoparticles has been developed by chemists for biomedical applications to meet imaging and targeting needs. In parallel, adoptive T therapy with chimeric antigen receptor engineered T cells (CAR T cells) has recently held great promise in B-cell malignancy treatments thanks to the development of anti-CD19 CAR T cells. Indeed, CD19 is a reliable B cell marker and a validated target protein for therapy. In this perspective article, we propose to discuss the advantages, limits and challenges of nanoparticles and CAR T cells, focusing on CD19 targeting objects: anti-CD19 nanoparticles and anti-CD19 CAR T cells, because those genetically-modified cells are the most widely developed in clinical setting. In the first part, we will introduce B cell malignancies and the CD19 surface marker. Then we will present the positioning of nanomedicine in the topic of B cell malignancy, before exposing CAR T technology. Finally, we will discuss the complementary approaches between nanoparticles and CAR T cells.

KEY WORDS

Nanoparticles, CD19, chimeric antigen receptor, T cell, B cell, cell therapy

OUTLINES

INTRODUCTION

A hematological malignant cell is defined as a hematopoietic cell blocked at an early stage of differentiation and undergoing an uncontrolled clonal proliferation. So far, tremendous improvement in cancer treatment has been obtained thanks to the identification of therapeutic drugs, better molecular understanding of the onset and progression of malignancy, more sensitive detection of tumor cells, more effective follow-up of the disease, better management of adverse effects, optimization of protocol design… Many challenges are still to be undertaken. From the time a patient arrives to be diagnosed to the moment he is cured, physicians and medical staff encounter at least the following issues: the early identification of the tumor, the imaging of malignant cells (where are localized the malignant cells? Is that the primary tumor or a metastasis?), the delivery of therapeutic drugs and avoidance of adverse effects on non-malignant cells (sometimes minimizing the risk of generation of a secondary cancer), and finally the identification of residual cells that could ultimately be at the origin of refractory cancer or relapse.

The chimeric antigen receptor (CAR) T cell therapy is a revolutionary approach of targeted immunotherapy to treat cancer. In CAR T cell therapy, the therapeutic effector is a genetically modified cell. CAR T cell therapy may not yet be poised to overtake chemotherapy as the standard of care, however, it is looking as a promising treatment for certain patients with no other feasible therapeutic option, such as in relapsed or refractory leukemia. An alternative research approach for the treatment of cancer is offered by nanoparticles, which have been proposed as carriers for drug encapsulation in the 60's. Since then, a variety of organic and inorganic nanoparticles, with sizes ranging from *circa* 5 nm to 200 nm, have been designed for a wide range of applications including targeted drug delivery and imaging, thus boosting the activity of nanomedicine, with some remarkable results particularly in the field of cancer diagnosis and therapy.

In this perspective article, we will propose to discuss the challenges of nanoparticles and CAR T cells in the context of hematological malignancies. We will focus on CD19 targeting objects: anti-CD19 nanoparticles and anti-CD19 CAR T cells because those genetically modified cells are the most widely developed in clinical setting.
In the first part, we will introduce B cell malignancies and their CD19 surface marker, then we will present the positioning of nanomedicine in the topic of B cell malignancy, before exposing CAR T technology. Finally, we will discuss the complementary approaches between nanoparticles and CAR T cells. From the biological point of view, anti-CD19-grafted nanoparticles and anti-CD19 CAR T cells target the same B cell lineage. From the therapeutic perspective, nanoparticles and CAR T cells approaches share common objectives: the optimization of therapeutic effect on target cells and the minimization of adverse effects. However, the mechanisms of action are different **(see the graphical abstract)**. It seems reasonable to conceive that nanoparticles could play a significant role for the potentiation of, and the cooperation with CAR T cell therapy in the future.

1 CD19, A B CELL RESTRICTED SURFACE PROTEIN AND A RELIABLE MARKER OF B CELL MALIGNANCIES

1.1 THE FUNCTIONS OF B LYMPHOCYTES

B cells (also named B lymphocytes) achieve multiple functions that explain their central role in the immune system **(Figure 1)**. Their main role is the production of antibodies to identify and neutralize pathogens. The binding of a B lymphocyte to an antigen triggers an initial step of multiplication and differentiation either into plasma cell which secretes antibodies or into memory B cell. Besides their role in humoral immunity, B cells are involved in cytokine production (*e.g.* IFNγ, IL6, IL10), antigen presentation to T cells, wound healing, cytokine balance for the differentiation between T lymphocytes (Th1 and Th2 cells), but also in the transplant rejection (review in (LeBien and Tedder, 2008)).

B cells undergo differentiation, from hematopoietic stem cells to plasma cells or memory B cells, through a series of stages characterized by the orderly rearrangement and expression of immunoglobulins genes including CD19 **(Figure 1)**. The development of B cells is also distinguished into different stages by the sequential expression of different transcription factors that induce immunoglobulin gene recombination and the expression of specific surface phenotypes. The onset of B cell lineage occurs in the bone marrow until the immature stage, then mature B cells move into the periphery (*i.e.* out of the bone marrow) (Zhu and Emerson, 2002).

1.2 B CELL MALIGNANCIES

B cell malignancies are hematological cancer characterized by uncontrolled proliferation of B lymphocytes blocked along their differentiation process. B cell malignancies are classified as leukemia (which develops in the bone marrow and disseminates into the body), lymphoma (a cancer of the lymphatic system characterized by the development of a cancer cells in lymph nodes) and myeloma (cancer of mature B lymphocytes in the bone marrow) (review in (Wang et al., 2012)). B cell malignancies represent 4% of all cancers in adults and 40% of all cancers in children. The clinical outcomes of these cancers under standard chemotherapy depend on the type of B cell malignancies. For instance, children with B-Acute Lymphoblastic Leukemia (ALL) have an overall good prognosis, but some of them are refractory to chemotherapy or develop multiple relapses and have a poor prognosis (review in (Park et al., 2016)). Relapsed or refractory B cell ALL in adults are associated with a poor prognosis (review in (Geyer and Brentjens, 2016)).

1.3 THE SURFACE PROTEIN CD19: A VALIDATED TARGET PROTEIN FOR THERAPY

1.3.1 CD19 structure and function

CD19 is a 95 kDa transmembrane glycoprotein of the immunoglobulin superfamily composed of an extracellular domain, a single transmembrane domain, and a cytoplasmic domain (Stamenkovic and Seed, 1988). CD19 belongs to the CD19 complex on the surface of B cells with CD21 and CD81 proteins **(Figure 2)**. CD19 activation induces two downstream pathways. The first cascade of activation is dependent on the B Cell Receptor (BCR). The BCR is composed of a membrane immunoglobulin and a signaling subunit composed of a heterodimer of immunoglobulin alpha and beta. The BCR plays a role as antigen receptor and CD19 is a co-receptor for BCR signal transduction (review in (Wang et al., 2012)). The second pathway depending on CD19 is independent of the BCR: the CD19 complex is able to bind activated complement fragment C3d and modulates BCR signaling (review in (Wang et al., 2012)).

1.3.2 Internalization of CD19 after binding to anti-CD19 antibody

CD19 proteins on the surface of each B lineage leukemia/lymphoma cells are rapidly internalized upon ligation with anti-CD19 antibodies or immunoconjugates (Uckun et al., 1988; Yan et al., 2005), and are ultimately taken up by lysosomes (Carter, 2006 ; Gerber et al., 2009; Hong et al., 2015).

1.3.3 Cells that express CD19

CD19 is a B cell-specific protein expressed early in B cell ontogeny (Stamenkovic and Seed, 1988) **(Figure 1)**. CD19 transcripts are restricted to members of the B cell lineage and are not expressed in other hematological lineages including normal myeloid, erythroid, megakaryocytic, or multilineage bone marrow progenitor cells (Uckun et al., 1988). CD19 protein is found on the surface of B cells from the proB cell stage until plasma cell differentiation of the B lineage (Tedder et al., 1994). Several hundred thousand CD19 proteins can be found on the surface of each B-lineage leukemia/lymphoma (Uckun et al., 1988)(review in (Li et al., 2017)). All resting B cells display CD19 antigens, and CD19 expression persists upon activation, but is lost upon further differentiation to immunoglobulin-secreting plasma cells (Stamenkovic and Seed, 1988). CD19 is also more abundant in pre-B cell lines and less abundant in plasmacytomas (Stamenkovic and Seed, 1988). Almost all early B cell malignancies show CD19 expression at normal to high levels: 80% of ALL, 88% of B cell lymphomas and 100% of B cell leukemias (review in (Wang et al., 2012)). However its expression decreases in myeloma cases (review in (Wang et al., 2012)).

1.3.4 CD19 as a target for therapy

Twenty years ago, CD19 was already proposed as a « suitable target for immunotoxin-mediated treatment of aggressive forms of B cell lymphomas and leukemia that responds poorly to conventional chemotherapy» (Uckun et al., 1988). Currently, CD19 antibody-based therapy has become reality to treat B cells malignancy. In the 2010's, various strategies harnessing the potential of targeting B cells restricted to CD19 antigen were in development: antibody-drug conjugate, Fc-engineered human CD19 antibody with antibody-dependent cell-mediated cytotoxicity, chimeric antigen receptor, etc. (Hammer, 2012). The most advanced anti-CD19 therapy is the Blinatumomab (BLINCYTO®, Amgen) (review in (Hammer, 2012)) (Goebeler and Bargou, 2016), a bispecific CD19-directed CD3 T cell engager (BiTE) antibody construct. Blinatumomab binds specifically to CD19 expressed on the surface of cells of B-lineage origin, and to CD3 expressed on the surface of T cells. It brings both cells in contact so that the activated T cells can kill the B cells. Blinatumomab is approved by the US Food-and-Drug-Administration (FDA) and the European Commission (EC) for the treatment of Philadelphia chromosome-negative relapsed or refractory B-ALL, in adults (USA and EC) as well as in children (USA only). Additionally, anti-CD19 antibodies are also in development for radioimmunotherapy in preclinical studies. ^{131}I-labeled anti-CD19 antibody has been largely explored for conventional ^{131}I radioimmunotherapy because antigen rapidly internalizes upon binding of antibody – resulting in catabolism and release of ^{131}I (Scheinberg and Strand, 1983). Moreover, ^{90}Y-particle-labeled anti-CD19 antibody has shown an efficacy comparable to ^{90}Y-labeled anti-CD20 antibody in radioimmunotherapy of mice with xenografts of human B lymphoma cell lines (Ma et al., 2002).

2 NANOMEDICINE IN THE TOPIC OF B CELL MALIGNANCY

A number of nanoparticles has been proposed by chemists for cancer diagnostics and therapeutics, as summarized **Table 1**. Organic nanoparticles, such as liposomes, oil-in-water emulsions or polymeric particles, are mainly used as carriers, whereas nanoparticles, such as superparamagnetic iron oxide nanocrystals or quantum dots, show interesting intrinsic properties for imaging and therapy.

2.1 NON TARGETING NANOPARTICLES FOR THERAPY AND IMAGING OF B CELL MALIGNANCY

Some anticancer encapsulation nanosystems have made their way to the market (Pattni et al., 2015). Liposomal formulations encapsulating drugs, such as doxorubicin, are commercialized under the name of Myocet, Doxil, Lipodox and Caelyx. Related to hematological malignancy, a phase III clinical trial is open for a liposome combinational delivery of two cytotoxic drugs (cytarabine and daunorubicin) for high risk acute myeloid leukemia (clinicaltrials.gov identifier NCT01696084) (Shi et al., 2017). With the ultimate goal of achieving both spatial and temporal control of drug delivery, nanocarriers have evolved from the mere "sustained" release to "triggered" release **(Figure 3)**. Indeed, in cancer, abnormal local conditions, such as pH, enzymatic activity or concentration in reactive oxygen species, can trigger the delivery of the drug. In addition to these endogenous signals, nanocarriers can also release their load on the effect of applied light, ultrasounds or a magnetic field (Bhattacharya et al., 2016; Kamaly et al., 2016).

In the topic of B cell malignancy, only few nanoparticles-based therapies are in development (Stephenson and Singh, 2017) (Shi et al., 2017). Among all the recent clinical-stage nanomedicines (Shi et al., 2017), a phase II clinical trial is open to evaluate a liposome, carrying a DNA oligonucleotide against the anti-apoptotic protein BCL-2, in relapsed or refractory B cell lymphomas (clinicaltrials.gov identifiers NCT01733238 and NCT02226965). Similar approaches of gene/RNAi delivery by silica-based nanoparticles to target B-cell lymphoma were described in mouse model (Martucci et al., 2016). Additionally, between 2011 and 2014, a phase I/II clinical trial was opened to evaluate the safety and tolerability of a poly(ethylenimine)-based transfecting polyplex carrying siRNA against eIF5A and a plasmid expressing a pro-apoptotic mutant of eIF5A under the control of a B cell specific promoter. This therapeutic agent was evaluated in relapsed or refractory B cell malignancies (clinicaltrials.gov identifier NCT01435720). Finally, an immunostimulant lipoplex composed of liposome and plasmid DNA (Chang et al., 2009) is in a phase I clinical trial in relapsed or refractory leukemia (clinicaltrials.gov identifier NCT00860522).

Tumors are currently diagnosed using various imaging modalities such as radiography, computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI) (Salem et al., 2014)(Navarro et al., 2017). However, the diagnosis of hematological malignancies can be challenging due to the diversity of imaging appearances and clinical behavior of these diseases (Navarro et al., 2017). Multimodal imaging approaches have been proposed to overcome these limitations, since they offer the ability to image with different resolutions and over different temporal and spatial scales. Cistaro *et al.* demonstrated the high potential of combined PET (using ^{18}F-fluorodeoxyglucose) and MRI (using paramagnetic contrast agent) in the evaluation of pediatric patients with ALL (Cistaro et al., 2017). By their work, they highlighted the real need of developing hybrid PET/MRI instruments and dual contrasts agents.

In line with that idea, a variety of nanoparticles has been designed to combine several imaging modes, multiple therapies, (*e.g.* photothermal therapy and conventional chemotherapy) or imaging and therapeutic functions (theranostics) and therefore holds great prospects in cancer treatment (Riley and Day, 2017). Among others, our group has recently reported on a vesicular platform, with a shell of inorganic nanoparticles named Hybridosomes® (Sciortino et al., 2016). The large number of nanoparticles forming the shell is a clear advantage for imaging applications, since an enhanced contrast is observed. Initially designed for MRI, these Hybridosomes® can not only be prepared from iron oxide superparamagnetic nanoparticles but also from any types and combinations of inorganic particles with imaging or therapeutic properties. Therefore, those multimodal nano-objects are suitable tools for multimodal imaging as well as theranostics. The feasibility of a theranostic approach has been demonstrated in acute myeloid leukemia patients where *in vivo* molecular imaging of CXCR4, a crucial protein involved in the retention of hematopoietic stem cells within the hematopoietic niche, has been achieved by means of positron emission tomography (Herhaus et al., 2016). However, as far as we know, there is still no open clinical trial using those combined strategies in B cell malignancies.

2.2 CD19-TARGETING NANOPARTICLES

The efficiency of imaging and treatment can be greatly improved by targeting specifically the malignant cells. As mentioned above, CD19 is currently the antigen of choice used to target B cells. Recently, CD19-targeting nanoparticles were designed for nanomedicine by grafting anti-CD19 antibody or its derivatives (Fab, $F(ab)_2$…) to the nanoparticles **(Figure 4)**. As an example, Cheng *et al.* produced liposomal doxorubicin targeted *via* anti-CD19 monoclonal antibody fragments: either the single-chain variable fragment (scFv), or the variable fragment (Fab), or the monoclonal antibody (mAb) **(Figure 4)**. The authors compared the efficacy of the three targeted constructs and concluded that the scFv single-chain variable fragment would be more suitable for development of immunotherapy for the following reasons: i) it contained less foreign peptides, ii) the production was easier, and iii) the cost of production was more economical thanks to the expression in bacterial systems (Cheng and Allen, 2008). Typically, four types of chemical functions from the antibody or its derivatives ($-NH_2$, -COOH, -SH, -carbohydrates) can be used for covalent grafting to the nanoparticle. The use of spacers such as PEG derivatives lowers the risk of antibody inactivation (Chen et al., 2016; Manjappa et al., 2011)(Nguyen et al., 2010)(Hong et al., 2015). Alternative strategies were also proposed, as the noncovalent strepatividin/biotin conjugation (Procko et al., 2014) (Dong et al., 2014).

2.2.1 Imaging with anti-CD19 nanoparticles

Few anti-CD19 grafted nanoparticles for *in vitro* imaging have been published so far. Nguyen *et al.* designed pegylated SERS (Surface Enhanced Raman Scattering) gold nanoparticles conjugated to human anti-CD19 antibody that showed specific *in vitro* targeting towards chronic lymphocytic leukemia (CLL) (Nguyen et al., 2010; Walker et al., 2012). The functional SERS nanoparticles were composed of a gold core onto which a reporter dye was adsorbed. The signals were detected by dark-field microscopy and Raman spectrometry and showed no interference with conventional fluorescent stains used in histology. Ramos B cells labeling through anti-CD19 mediator was demonstrated by Dong *et al.* by grafting an anti-CD19 antibody onto Ag@SiO_2 core-shell nanoparticles (Dong et al., 2014). In this study, the authors monitored the metal-enhanced fluorescence of a reporter (rhodamine B) adsorbed on the surface of the nanoparticles. However, to the best of our knowledge, *in vivo* imaging using anti-CD19-grafted-nanoparticles has not been reported yet.

2.2.2 Therapy with anti-CD19 nanoparticles

2.2.2.1 *Chemotherapy: drug delivery*

Nanoparticles decorated with anti-CD19 have already been reported as effective carriers for drug delivery on *in vitro* models and preclinical studies **(Table 2).** Doxorubicin, an inhibitor of topoisomerase involved in DNA synthesis, is frequently the drug of choice for proof-of-concept, as the cytotoxic effect of this drug is well demonstrated on B cells. A doxorubicin loaded immunoliposome targeting B lymphocytes showed a 6-fold more cytotoxic *in vitro* activity on B cells than non-targeted liposomes (Lopes de Menezes et al., 1998). Similar results were observed *in vivo* with an improved survival of mice injected with anti-CD19-doxorubicin-liposomes compared to non-targeted liposomes or free doxorubicin treatments (Lopes de Menezes et al., 1998). Doxorubicin was also encapsulated into block-copolymer nanoparticles grafted with anti-CD19. A clathrin-dependent internalization pathway was identified, suggesting that the physiological internalization pathway of CD19 was conserved. In comparison to the administration of free doxorubicin, both improved *in vitro* apoptosis of CD19 positive cells and better survival of treated mice were demonstrated (Krishnan et al., 2015). *In vivo*, mice xenografted with B cells and exposed to anti-CD19-liposomes containing doxorubicin or vincristine demonstrated a higher cell cytotoxicity and showed a longer survival time than mice exposed to free drug (Sapra and Allen, 2004). Those anti-CD19-liposomes showed *in vitro* a greater binding, a more effective internalization and an equivalent cytotoxicity on B cells compared to anti-CD20-liposomes (Sapra and Allen, 2004).

Other inhibitors of B-cells than doxorubicin or vincristine have also been evaluated and incorporated into nanoparticles. As an example, the C61 molecule (1,4-bis (9-*O*-dihydroquinidinyl) phthalazine/hydroquinidine 1,4-phathalazinediyl diether) was identified as a potent inhibitor of the cytoplasmic protein SYK (spleen tyrosine kinase), an important regulator of B cell apoptosis **(Table 2)**. Myers *et al.* demonstrated that a liposomal nanoparticle formulation entrapping C61 and decorated with anti-CD19 caused *in vitro* the apoptosis of pre-B ALL cells, twice more than the non-decorated liposomes (Myers et al., 2014). Immunocompromised NOD/SCID mice were then xenografted with pre-B ALL cells, and injected with C61-liposomes decorated with anti-CD19. Tumor cell viability decreased and mice did not develop leukemic splenomegaly, thus showing a better therapeutic efficacy than irradiation with 2Gy γ-rays. In addition, the combination of C61 loaded anti-CD19-liposomal nanoparticles, with exposure to low dose of radiations, caused the abrogation of B leukemia in engrafted mice (Myers et al., 2014).

In addition, multifunctional immunoliposomes grafted with several antibodies were shown to exhibit higher selectivity, greater binding affinity, and enhanced apoptosis induction of B-CLL cells (Woyach et al., 2014). Yu *et al.* also proposed a dual ligand conjugation on immunoliposomes (Yu et al., 2013). The authors first evaluated the level of expression of CD19, CD20 and CD37 antigens in several B cell lines and primary B-CLL cells, and found comparable level for CD19 and CD37. They also calculated the internalization rate of the three antibodies in lymphoma cells (Raji cells) and confirmed the choice of anti-CD37 as the primary ligand for specific targeting of B cells. Then they measured the binding efficacy of single or mixtures of anti-CD19, anti-CD20 and anti-CD37 on B-CLL cells isolated from patients. Greater binding efficacies occurred with dual combinations of anti-CD19 and anti-CD20, with anti-CD37 antibody. The antibody ratio was finally optimized to improve this synergetic effect.

Note that the combination of several specific antibodies is also a promising strategy to overcome the variability in the expression of target antigens among patients. In this context, hydroxychloroquine, an anti-malaria and anti-rheumatic drug, has been encapsulated in order to overcome pharmacokinetic obstacles and to deliver a larger amount of this apoptotic drug into B-CLL cells from patients. As an example, Mansilla *et al.* encapsulated hydroxychloroquine in PEG-PLGA nanoparticles mono-functionalized by anti-CD19 antibody or bi-functionalized by anti-CD19 and anti-CD20 antibodies (Mansilla et al., 2010). The authors showed a significant induction of apoptosis of B-CLL cells with mono- or bi-functionalized nanoparticles compared to non-functionalized nanoparticles.

2.2.2.2 *Nanoparticle-based immunotherapy*

An innovative strategy consists in using nanoparticles exposing antibodies in order to stimulate the production of lymphocytes, or even to bridge malignant cells to killer T cells **(see the graphical abstract)**. Schütz *et al.* designed nanoparticles termed antigen-specific T cells redirectors (ATR). The ATR nanoparticles were conjugated to two antibodies, an anti-TCR and an anti-CD19. The ATR nanoparticles provided a physical proximity between T cells and tumor cells, and redirected T cells to kill tumor cells (Schütz et al., 2016). *In vivo* assays on mice xenografted with lymphoma cells and injected with ATR nanoparticles showed smaller tumors and an improved survival compared to control mice.

3 CD19-TARGETED CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS IMMUNOTHERAPY

An alternative to nanoparticles for targeting tumor cells is to take advantage of other cells. For years, most of hematological neoplasms have been treated by hematopoietic stem cell transplantations. The transplanted allogeneic hematopoietic stem cells kill residual malignant cells by a graft-versus-tumor effect. This cell therapy approach, used to fight leukemia, lymphoma or myeloma, leads to either remission or immune control of the malignancy; however, some patients relapse. On the other hand, many therapeutic approaches tend to modulate the immune response to eliminate tumor cells. Immunotherapy has marked the past years by generating extraordinary advances in clinical applications for cancer treatment.

Cell immunotherapy harnesses the power of both cell therapy and immunotherapy, and is at the origin of tremendous clinical progresses in the past decade (Ramachandran et al., 2017). For the purpose of the review, we will focus on CD19 antibody-based cell immunotherapies that target B cell neoplasms.

3.1 IMMUNOTHERAPIES: ANTIBODY-BASED AND ADOPTIVE CELLULAR THERAPIES

3.1.1 The concept of CAR T cell: retargeting a cytolytic immune cell by genetic-modification to eliminate a tumor cell

T lymphocytes are cells that play a central role in cell-mediated immunity. Different subsets of T cells achieve cytolytic, regulatory or memory roles. Genetically retargeting T cells against tumor surface antigens to trigger cytotoxic mechanisms against malignant cells is one of the principles of adoptive cell therapy. More precisely, the engineering of T cells to express a chimeric antigen receptor (CAR) is the most common gene-modifying strategy that is being investigated. CARs are synthetic receptors that direct the genetically engineered T cells against tumor surface antigens, for instance CD19 antigen. Adoptive cell therapy using gene-modified T cells has emerged as an exciting therapeutic approach for the treatment of cancer (Porter et al., 2011; Kochenderfer et al., 2012 ; Brentjens et al., 2013).

3.1.2 The main biological challenges for an effective antibody-based adoptive cellular therapy

Conceptually, many challenges should be faced to achieve an *in vivo* therapeutic efficacy. The first one is that CAR T cells must be able to persist *in vivo*, and then undergo cellular expansion (Grupp et al., 2013). They will also have to infiltrate tumor tissues (in case of solid tumors), then to engage their target antigen expressed on tumor cells, and finally, to exert their cytolytic, proliferative, and cytokine secretory activities within the tumor microenvironment to eliminate malignant cells (review in (Beatty and O’Hara, 2016)).

Adoptive T cell therapy with chimeric antigen receptor engineered T cells (CAR T cells) has shown substantial clinical results against B cell malignancies (Porter et al., 2011; Kochenderfer et al., 2012 ; Brentjens et al., 2013). The fact that CAR T cell therapy approach has proven to be of some effectiveness across a range of hematological malignancies (Gill and June, 2015) may be partly explained by the choice of a relevant target antigen (for instance CD19) and by the fact that those malignancies reside in the natural sites that adoptively transferred T cells naturally invade (review in (Newick et al., 2016).

3.1.3 The choice of a relevant target antigen: CD19 gene therapy

As mentioned previously, CD19 is a reliable target antigen for antibody-based therapy (review in (Hammer, 2012)(Li et al., 2017)). More than half of all CAR-modified T cell studies in hematological malignancies have targeted CD19 antigen (review in (Beatty and O'Hara, 2016)). CD19-specific CAR T cells have demonstrated potent activity in B cell ALL and lymphomas including CLL and non-Hodgkin lymphoma (Porter et al., 2011 ; Grupp et al., 2013; Maude et al., 2014 ; Davila et al., 2014 ; Lee et al., 2015 ; Brudno et al., 2016 ; review in Beatty and O'Hara, 2016).

3.1.4 The role of CAR: conferring T cell the ability to persist and expand *in vivo* and to exert cytolytic activity

3.1.4.1 *Design of CAR*

The chimeric antigen receptor (CAR) is composed by two main modules: (i) an extracellular component that recognizes a cell surface protein (*e.g.* CD19) (this extracellular moiety is a single-chain variable fragment (scFv) derived from an antibody) linked to (ii) an intracellular component consisting in T cell signaling domains of the T cell receptor (e.g. CD3ζ) including co-stimulatory domains (e.g. CD28, or 4-1BB) involved in T cell activation **(Figure 5)** (review in (Beatty and O'Hara, 2016) and (Geyer and Brentjens, 2016)). The extracellular component is responsible for redirecting T cell specifically to the human tumor antigen whereas the intracellular component sustains T cell activation, supporting cell expansion and cytokine release resulting in cytolytic activity.

Intense work is done to optimize each module: the extracellular component which acts as the target-binding domain of the CAR, the hinge region connecting extracellular and intracellular component (Hudecek et al., 2013), and the intracellular component for an effective T cell proliferation and differentiation to mature effector T cells. The successive generations of CD19 CAR T differ in the number and origin of the intracellular co-stimulatory domains **(Figure 5)** (*e.g.* 4-1BB or CD28) (Savoldo et al., 2011 ; Porter et al., 2011 ; Maude et al., 2014 ; Park et al., 2016).

3.1.4.2 *Mechanism of action of CAR T cells*

The binding of the anti-CD19 scFV to CD19 antigen of tumor cell surface (the resulting complex is named the immune synapse) sends a signal through the CAR to the effector T cell. This signal results in the activation of the T cell and in the release of soluble molecules, perforin, granzyme and pro-apoptotic ligands, that kill the tumor cells. Additionally, activated T cells secrete proinflammatory cytokines (*e.g.* interferon IFN-γ, and IL-2), amplifying the immune response (Davenport et al., 2015) (Geyer and Brentjens, 2016), and leading to the expansion of CAR T cells. The range of *in vivo* expansion of CAR T cells has been reported between 100- to 10 000- fold (Grupp et al., 2013).

3.2 CURRENT CLINICAL OUTCOMES, BENEFITS AND LIMITATIONS OF CD19 CAR T THERAPY

3.2.1 Clinical outcomes

Many patients go into remission with standard chemotherapy for B cell malignancies. However, children and adults with relapsed or refractory B cell ALL have a poor prognosis. Substantial clinical efficacy has been demonstrated with a therapy based on CAR-modified T cells targeted to CD19. Approximately 70% of patients underwent complete or at least partial response to treatment with chimeric antigen receptor CAR-modified T cells targeted to CD19 (Porter et al., 2011; Kochenderfer et al., 2012 ; Brentjens et al., 2013 ; Grupp et al., 2013 ; Maude et al., 2014 ; Davila et al., 2014 ; Lee et al., 2015). Results are less impressive with CLL or with B cell non-Hodgkin lymphoma but still subsets of patients show significant benefits (review in (Geyer and Brentjens, 2016). Clinical trials are ongoing for multiple myeloma.

3.2.2 Advantages

In vivo expansion and persistence of CAR T cells is a clear determinant of clinical benefit (Grupp et al., 2013 ; Porter et al., 2015 ; Beatty and O'Hara, 2016)**.** In addition, the natural trafficking of CAR T cells within the blood, lymph nodes, and bone marrow where they encounter malignant cells also favors the efficacy of the therapy (Beatty and O'Hara, 2016). Furthermore, it appears that the accessibility to malignant cells is less hindered by the tumor microenvironment in those tissues compared to solid tumors (Geyer and Brentjens, 2016 ; Newick et al., 2016).

3.2.3 Limitations

3.2.3.1 *Genetic modification of autologous T cells*

First, each patient is infused with his own T cells. This specificity limits any large-scale manufacturing process and anticipated stocks. Then, autologous T cells are subjected to genetic modifications by retrovirus, lentivirus or non-viral gene transfer followed by *in vitro* stimulation. Currently, the complicated and individualized production of autologous CAR T cells may be one, among others, of the bottlenecks that reduce accessibility to this personalized therapy to many people. Some strategies using universal T cells (*i.e.* that do not come from the patient) are also in development. Suboptimal expression of the CAR at the surface of CAR T cells may also limit the benefit of CAR T cell therapies. Recently, Eyquem *et al.* have proposed that directing a CD19-specific CAR to the T cell receptor α constant (*TRAC*) locus not only results in uniform CAR expression in human peripheral blood T cells, but also enhances T cell potency, with edited cells vastly outperforming conventionally generated CAR T cells in a mouse model of ALL (Eyquem et al., 2017).

3.2.3.2 *The need of lymphodepletion for the patient*

The purpose of chemotherapy, whose objective is to achieve lymphodepletion prior to CAR T cells infusion, is to create a more favorable environment for CAR T cells. Most studies corroborated the notion that host lymphopenia (*i.e.* a low number of lymphocytes in the blood) facilitates the expansion of adoptively transferred T cells. Whether lymphodepletion might further enhance the activity of CAR T cells in this setting remains unclear (Brudno et al., 2016 ; Turtle et al., 2016). To date, induction of lymphodepletion prior to infusion of CAR T cells continues to be often incorporated in clinical trials using CAR T cells.

3.2.3.3 *Toxicity for the patient*

The medical community will have to overcome clinical challenges related to CD19-targeted CAR T cells (Geyer and Brentjens, 2016; Park et al., 2016). Major side-effects, particularly cytokine release syndrome, neurological toxicities, and B cell aplasia have been reported in all clinical trials using CD19-targeted CAR T cells. The cytokine release syndrome is a severe inflammatory response syndrome that appears within the hours to days following CAR T cell infusion. Clinical features include fevers, muscle pain, malaise, and, in more severe cases, hypoxia, hypotension, and occasionally renal dysfunction and coagulopathy. The cytokine release syndrome is characterized by elevation of pro-inflammatory cytokines (e.g. IL-6) and T cell activation and expansion. Tumor burden is positively correlated with the risks of severe cytokine release syndrome and neurotoxicity (Brentjens et al., 2013) (Turtle et al., 2016). The cytokine release syndrome can be life-threatening and requires intensive supportive care. Mitigating strategies to reduce cytokine release syndrome frequency and severity comprise anti-IL-6 receptor antibody, steroids, and possibly a protocol-specified algorithm to potentially start pre-emptive treatments (Maude et al., 2014; Lee et al., 2014 ;Turtle et al., 2016; Ruella et al., 2017).

Reversible neurologic toxicity has been observed after CAR T cell infusion, including delirium, seizure-like activity, confusion, word-finding difficulty, or aphasia.

Finally, CD19-targeted CAR T cells therapy shows “on-target, off-tumor” toxicity that generates B cell aplasia (Porter et al., 2011 ;Grupp et al., 2013; Maude et al., 2014).

Limiting B cell aplasia for CD19-targeted CAR T cells has been successfully managed with intravenous immunoglobulin replacement therapy (Frey and Porter, 2016). Novel approaches to limit B cell aplasia are under investigation as the use of antigen-specific inhibitory CAR to protect normal B cells (Fedorov et al., 2013).

3.2.3.4 *CD19-antigen escape*

Loss of expression of the CD19-target antigen resulting in an antigen escape (*e.g.* CD19-negative relapse) may limit the benefit of CD19 CAR T cells therapy (Grupp et al., 2013). Tumor antigen escape has emerged as a main challenge for the long-term disease control (review in (Wang et al., 2017;Velasquez and Gottschalk, 2017)). Studies are going on to understand the mechanism of loss of CD19 expression and overcome this difficulty. Braig *et al.* reported emergence of CD19-relapses due to CD19 mRNA splice variants (Braig et al., 2017). Zah *et al.* proposed a design of bispecific CARs that triggered robust cytotoxicity against target cells expressing either CD19 or CD20 and controlled both wild-type B cell lymphoma and CD19 mutants with equal *in vivo* efficacy (Zah et al., 2016).

3.2.3.5 *Infused dose, composition, and control of expansion and function of CAR T cells*

So far, the different clinical trials have not led to the identification of a clear correlation between higher CAR T cell infused dose and greater efficacy or CAR T cell persistence (Porter et al., 2011 ; Grupp et al., 2013; Maude et al., 2014 ; Davila et al., 2014 ; Lee et al., 2015 ; Brudno et al., 2016) (review in (Park et al., 2016; Geyer and Brentjens, 2016)). Importantly, the efficacy of CAR T cells relies on their activation in response to CD19 antigen and expansion *in vivo,* making the magnitude of their reactivity unpredictable (Grupp et al., 2013). For instance, anti-CD19 CAR T cells have been shown to proliferate in excess of 100,000-fold in some patients, ultimately accounting for over 50% of circulating lymphocytes. The lack of control over CAR T cells activation and expansion *in vivo* is a limit to predict the therapeutic response.

Multiple parameters provide clues to explain this unpredictability. The composition of the infused therapeutic agent is source of variability. So far, CAR T cells are generated from autologous T cells, making the received therapeutic agent different for each patient (Sommermeyer et al., 2016) (Turtle et al., 2016). In preclinical studies, where mice were injected with a same pool of CAR T cells, a better correlation between the infused dose and the xenografted mouse survival was observed (Sommermeyer et al., 2016). More precisely, the variability of CAR T cells encompasses extrinsic parameters, from the efficacy of genetic modification to the expression of the CAR at the surface of CAR T cells, but also intrinsic interindividual parameters including composition of CD4+ and CD8+ T cells. In CAR T therapy, CD4+ CAR T cells are responsible for cytokine production whereas CD8+ CAR T cells trigger direct antitumor effects. The ratio of CD4+/CD8+ CAR T cell subsets may be of importance in the balance between efficacy and toxicity (Park et al., 2016). In most reported trials, patients received CAR T products comprising random compositions of CD4+ and CD8+ T cells. In contrast, Sommermeyer *et al.* and Turtle *et al.* showed that CAR T cell products generated from defined T cell subsets (1:1 ratio of CD4+ and CD8+ CAR T cells) can provide uniform potency compared with products derived from unselected T cells and induce complete remission without a high rate of toxicity in patients with a high tumor burden (Sommermeyer et al., 2016 ; Turtle et al., 2016). Approaches to limit expansion and activation are also underway. Rodgers *et al.* propose a method to control CAR T cells using peptide-engrafted antibody-based molecular switches that act as a bridge between the target cell and CAR T cells (Rodgers et al., 2016).

Interindividual variation in response to the treatment can also be attributed to difference in lymphodepletion between each patient, or to difference in immunological clearance that will impact the persistence of the infused and expanded CAR T cells.

Altogether, the optimal dose and composition of the CAR T cell product remain under development in order to achieve a better predictability in response to the therapeutic agent and to balance toxicity and efficacy.

4 PERSPECTIVES: HOW NANOPARTICLES AND CAR T CELL THERAPY COULD BE COMPLEMENTARY?

4.1 MULTIMODALITY

The efficacy of CAR T cell therapy relies on the multimodality of the therapeutic response. CAR T cells target tumor cells, trigger cytolytic activity, and ensure their own expansion. We can envision that the future of nanomedicine will benefit from the same feature: the multimodality. It is clear that nano-objects, and among them Hybridosomes® (Sciortino et al., 2016), can address many of the challenging issues of hematological cancer diagnosis and therapy. In particular, nanoparticles could play a significant role for the potentiation of, and the cooperation with CAR T cell therapy. Their complementarity (in terms of function, distribution and time of administration) can be envisioned to fulfill at least three objectives: (i) to track malignant cells and

CAR T cells to monitor their biodistribution and expansion, (ii) to increase tumor accessibility, and (iii) to manage CAR T cell toxicity and modulate the expansion of CAR T cells.

4.2 TO TRACK MALIGNANT AND CAR T CELLS

Since the proof-of-concept of CAR T cells has been validated, current developments include the control of cell expansion or avoidance of CD19 escape. There is a need for noninvasive tracking of the transfused T cells in patients to determine their biodistribution, viability, and functionality (review in (Liu and Li, 2014)). Several strategies based on nanoparticle contrast agents have been proposed using either *ex vivo* preloaded nanoparticles on CAR T cells, or *in vivo* administration of nanoparticles after CAR T cell infusion. For instance, in mouse model, CAR T biodistribution has been monitored through radiolabeled-nanoparticles or contrast-agent-nanoparticles loaded into CAR T cells prior to cell infusion (Bhatnagar et al., 2013;Bhatnagar et al., 2014).

Furthermore, detecting the localization of tumor cells is of particular importance in the case of hematological cancer, since hematological malignant cells are intrinsically disseminating. In addition, *in situ* imaging alternatives to the invasive sampling of bone marrow are desirable for diagnosis and for residual disease follow-up. By proposing efficient targeting contrast agents, nanomedicine can greatly improve the diagnosis, and beyond, the determination of localization of tumor cells (Kobayashi et al., 2005).

4.3 TO IMPROVE TUMOR ACCESSIBILITY

A recent statistical review of the literature revealed that less than 1% of the injected nanoparticles systemically reaches the malignant cells in solid tumors, compromising their translation into clinical use (Wilhelm et al., 2016). This figure is due both to nanoparticle uptake by the immune system, and to their poor mobility into the tumor microenvironment. Although hematological malignancies differ from other solid tumors, some limitations of the CAR T therapy due to limited access to specific accumulation sites may be observed as well. According to cancer type, hematological malignant cells originate from the bone marrow (*e.g.* leukemia, myeloma) or lymph node (*e.g.* lymphoma), and infiltrate blood stream and solid tissues. The bone marrow niche is a very complex environment essentially composed of a dense network of small arterioles and sinusoids, and of various cell types within an extracellular matrix (Wu et al., 2008) (Morrison and Scadden, 2014) (Gattazzo et al., 2014)(Schepers et al., 2015). Leukemic stem cells, as well as hematopoietic stem cells, are dependent on those cells and extracellular components for their emergence, homing and survival. Disruption of those interactions participates in the efficacy of the therapy.

The combination of the specific properties of CAR T cells and nanoparticles seems promising to enhance the efficacy of treatments. Indeed, CAR T cells will guarantee longer circulation time in the blood stream and specific recognition of B cells, whereas nanoparticles can bring advantageous features such as degradation of the extracellular matrix, disruption of cell-cell interactions, or thermal stimulation. An advance in this direction was already reported in the literature. In mouse studies, Kennedy *et al.* used T cell as chaperones for gold nanoparticle delivery to enhance the efficacy of nanoparticle-based photothermal therapies and imaging applications by increasing accumulation at tumor site (Kennedy et al., 2011). Another innovative strategy, inspired by motile and invasive cells, would be the active enzymatic degradation of the tumor matrix by protease that can be associated with the nanotherapeutic system. For instance, iron oxide nanoparticles coated with collagenase were magnetically driven through *in vitro* extracellular matrix, at a rate similar to invasive cells (Kuhn et al., 2006). Other proteolytic surfaces include bromelain, an enzymatic complex belonging to the papain family and extracted from pineapple which contains a mixture of 9 proteases with distinct pH and enzymatic activities (Parodi et al., 2014). Local heating triggered by external sources can also be used to alter the tumor environment and enhance accessibility to malignant cells, based on gold nanoparticles (Gormley et al., 2013; Smith et al., 2015).

An alternative strategy would be the pretreatment with therapeutic nanoparticles prior to CAR-T infusion. In this line, nanoparticles targeting the bone marrow niche could also be utilized to specifically deliver high doses of lymphodepleting agents prior to CAR T infusion. Similarly, pre-treatment with drugs, specifically targeting the interaction of leukemic stem cells with their bone marrow niches, may be useful to mobilize those cells and render them more accessible to CAR T cells in the marrow or the blood stream. Among others, inhibitors of the adhesion molecule E-selectin, or inhibitors of the chemoattractant stromal-cell-derived factor 1 (SDF-1) could be proposed because leukemic stem cells are dependent on those molecules for their homing (Sipkins et al., 2005)(Krause and Scadden, 2015)(Schepers et al., 2015). Identification of additional specific factors in B cell malignancies could be of interest for mobilizing B cells and enhancing CAR T cell therapy, as exemplified by the role of CD44, or various selectins and their ligands in chronic myeloid leukemia or acute myeloid leukemia (Krause et al., 2006)(Jin et al., 2006)(Krause et al., 2013).

4.4 TO MANAGE TOXICITIES OF CAR T CELLS AND MODULATE THE EXPANSION OF CAR T CELLS

Major toxicity such as severe cytokine release syndrome is intrinsically related to CAR T efficacy, and current developments aim at controlling it. Current strategies to allow preferential removal of CAR T cells include genetic “safety switch” or drug sensitivity (review in (Ranganathan and Foster, 2016)). In this perspective, nanoparticles could be specifically designed to target CAR T cells, making possible a selective apoptosis of those cells or a selective removal of those cells. In this line, an innovative strategy related to hematological diseases is the magnetic sorting of sick cells, after attachment of a magnetic particle. In some cases, such as malaria, the intrinsic magnetic properties of infected cells even allow magnetic sorting of unlabeled cells (Zborowski and Chalmers, 2011). Nanoparticles targeting tumor cells or CAR T cells could be used to lower the tumor burden (lymphodepletion) before treatment or alternatively remove CAR T, after treatment or in case of excessive expansion of CAR T cells.

5 CONCLUSION

Nanomedicine and cell therapy are two fields that have grown in parallel. Yet, those approaches aim ultimately at common goals, to achieve long remission and ideally the cure of the patients. In this review, based on the example of developing tools to target B cell malignancy (mostly anti-CD19 nano-objects and anti-CD19 CAR T cells), we have discussed their specificity, limitations and potential complementarity. It appears that even if CART T cell therapy has revolutionized management of patients presenting poor prognosis B cell malignancy, improvements are needed, especially to predict the therapeutic response, to control the intensity and persistence of the treatment, to increase tumor accessibility of the therapeutic agent to leukemic stem cell niches, and to visualize residual leukemic clones, and thus prevent relapses. Therefore, therapeutic developments could benefit from nanoparticles advantages -mainly their multimodality combining imaging and loading capacity, their tendency to accumulate at tumor sites for solid tumors and their relative easiness to be produced- to fill those requirements.

TABLES

Table 1: Main chemical and physical properties of the different types of nanoparticles used in nanomedicine and their principal applications. Note that the given size corresponds to the primary nano-object. In the case of small nanoparticles (NP) such as dendrimers or quantum dots (QD), surface modification with PEG or other macromolecules result in larger dimension.

NP type	Size (nm)	Organic/Inorganic	Principal application
Liposome	30-500	organic	encapsulation
Polymer NP	10-200	organic	encapsulation
Polymersome	50-1000	organic	encapsulation
Dendrimer	< 10	organic	encapsulation / imaging
Solid Lipid NP (and emulsion based particles)	> 100	organic	encapsulation
Silica NP	all range	inorganic	encapsulation / imaging
Quantum dot	5-20	inorganic	imaging
SPION	5-100	inorganic	imaging
Au NP	5-100	inorganic	imaging / therapy
Hybridosome®	80-120	organic/inorganic	imaging / encapsulation / therapy

Table 2: Nanoparticles (NP) grafted with anti-CD19 antibody and their applications in nanomedicine.

Abbreviation: Ag Silver; Au: Gold; Chol: Choline; DOTAP: 1,2-dioleoyl-3-trimethylammoniumpropane; DOPE: dioleoylphosphatidylethanolamine; DSPE: Distearoylphosphatidylethanolamine; EggPC: Egg yolk phosphatidylcholine; HD37-CCH: Hybridomas HD37-c-myc-Cys-His5 scFv; HSPC: hydrogenated soy phosphatidylcholine; LNP: liposomal nanoparticle ; MHC-Ig: Major Histocompatibility Complex-Immunoglobulin; NHS: N-hydroxysuccinimide; PEG: Polyethylene glycol; PLGA: poly(lactic-co-glycolic acid); SERS: Surface Enhanced Raman Scattering; SiO2: Silicon dioxide ; SYK: Spleen Tyrosine Kinase; TCR: T cell receptor

NP type	Composition	Targeting agent	Size (nm)	Application	Reference
Liposome	PEG-DSPE	anti-CD19	100-120	doxorubicin ***carrier*** *140-160 µg/µmol of phospholipid*	*Lopes de Menezes et al., 1998*
Liposome	HSPC/Chol/ mPEG-DSPE	anti-CD19	90-110	doxorubicin ***carrier***	*Sapra and Allen, 2004*
Liposome	SM/Chol/ mPEG-DSPE	anti-CD19	110-130	vincristin ***carrier***	*Sapra and Allen, 2004*
Liposome	mPEG$_{2000}$-DSPE	anti-CD19 hd37-cch fragment	80-120	doxorubicin ***carrier***	*Cheng and Allen, 2008*
Liposome	EggPC/Chol/ PEG$_{2000}$-DSPE	anti-CD19 + anti-CD37 / anti-CD19 + anti-CD20 + anti-CD37	100	FTY720 ***carrier***	*Yu et al.,2013*
Liposome	DSPE-PEG$_{3400}$-NHS	mouse anti-CD19	~135	C61 ***carrier*** *9,4 mg/mL*	*Myers et al., 2014*
Polymer NP	PEG-PLGA	anti-CD19 / anti-CD19 + anti-CD20	~300	hydroxychloroquine ***carrier*** *165 µg/mg of polymer*	*Mansilla et al., 2010*
Polymer NP	EG$_{113}$CL$_{152}$TSU$_{25}$	anti-CD19	~ 60	doxorubicin ***carrier*** *72,1+/-6,4 µg/mg of polymer*	*Krishnan et al., 2015*
Inorganic	Au@PEG	human anti-CD19	60-80	SERS ***cell imaging*** *MGITC = Raman tag*	*Nguyen et al., 2010*
Inorganic	Ag@SiO$_2$	anti-CD19	100-140	Fluorescence ***cell imaging***	*Dong et al., 2014*
Inorganic	Iron oxide@dextran	pep-MHC-Ig dimer or anti-TCR-specific with anti-human CD19	~50	***Targeting*** *Redirect T cells against tumor cells*	*Schütz et al., 2016*

FIGURE LEGENDS

Figure 1: B cell development and differentiation

B cell development begins in bone marrow and progresses through pre pro B cell, pro B cell, small pre B cell, large pre B cell and immature pre B cell. B cell locates within the circulatory system from mature B cell stage. The CD19 protein is expressed from pro B cell stage.

Figure 2: CD19 signaling complex and activation pathways

(A) Schematic representation of the CD19 signaling complex. The CD19 complex is composed of CD21, CD81 and CD19 transmembrane proteins. CD19 possesses an intracellular tail with multiple tyrosine-kinase residues involved in signal transduction. (B) The first pathway of CD19 activation is dependent on the B cell receptor (BCR): it is a co-receptor for BCR signal transduction. The second pathway is independent of the BCR: the CD19 complex is able to bind activated complement fragment C3d and modulates BCR signaling (Figure adapted from (Wang et al., 2012)).

Figure 3: The two main modes of controlled release from carrier nanoparticles

Sustained release can be operated by biodegradable carriers, most often polymeric, which are progressively eroded, or by porous (silica, polymer...) particles. Trigger-activated particles deliver their load at once, upon activation by an endogenous or exogenous trigger.

Figure 4: Natural and engineered antibody formats, and functional groups available for covalent labeling or bioconjugation

(A) Schematic representation of full monoclonal antibody (mAb) of 150 kDa and its scFv derivative of 55 kDa. Functional groups present on the antibodies and available for covalent labeling or bioconjugation are schematically represented (amine groups, carboxylate groups, thiol groups and carbohydrate residues). Fab: variable region; Fc region: constant region; VL: Variable Light chain; VH: Variable Heavy chain; CL: Constant Light chain; CH: Constant Heavy chain.

(B) Comparison between mAb and its derivatives in terms of size, pharmacokinetics, valency/specificity and strengths/weaknesses.

Figure 5: Chimeric antigen receptor (CAR)

Chimeric antigen receptor (CAR) of second generation is composed of a targeting element (here the single chain variable fragment (scFv) of anti-CD19), a transmembrane domain, a co-stimulatory domain and a signaling domain.

LIST OF ABBREVIATIONS

ALL: acute lymphoblastic leukemia
Ag: Silver
Au: Gold
BCR: B cell receptor
B-ALL: B cell acute lymphoblastic leukemia
CAR: chimeric antigen receptor,
CL: Constant Light chain;
CH: Constant Heavy chain
Chol: Choline
CLL : chronic lymphocytic leukemia
DOTAP: 1,2-dioleoyl-3-trimethylammoniumpropane
DOPE: dioleoylphosphatidylethanolamine
DSPE: Distearoylphosphatidylethanolamine
EC : European Commission
EDC: (1-ethyl-3-(3- dimethyl-aminopropyl)carbodiimide hydrochloride
EggPC: Egg yolk phosphatidylcholine
EPR : Enhanced Permeation and Retention
Fab: variable region
Fc region: constant region
FDA : US Food-and-Drug-Administration
IFNγ : interferon gamma
IL6: interleukin 6
HD37-CCH: Hybridomas HD37-c-myc-Cys-His5 scFv
HSPC: hydrogenated soy phosphatidylcholine
LNP: liposomal nanoparticle
mAb: monoclonal antibody
MGITC: Malachite Green Isothiocyanate
MHC-Ig: Major Histocompatibility Complex-Immunoglobulin
MPS: mononuclear phagocyte system
MRI : Magnetic Resonance Imaging
MRI/CT : magnetic resonance imaging/ computerized tomography
MRI/PET : magnetic resonance imaging/ positron emission tomography
NHS: N-hydroxysuccinimide
NP:nanoparticle
PEG: Polyethylene glycol
PLGA: poly(lactic-co-glycolic acid)
PVP: polyvinylpyrrolidone
QD: quantum dots
RES : reticuloendothelial system
SERS: Surface Enhanced Raman Scattering
SiO2: Silicon dioxide
siRNA:small interference RNA
SMCC: N-succinimidyl 4-(N maleimidomethyl)cyclohexane-1-carboxylate
SPDP: N-succinimidyl 3-(2-pyridylthio)propionate
SPECT: single, photon emission computed tomography
scFv: single-chain variable fragment
SYK: Spleen Tyrosine Kinase
TCR: T cell receptor

TEM: transmission electron microscopy
UCNPs : up-converting nanoparticles
VL: Variable Light chain
VH: Variable Heavy chain

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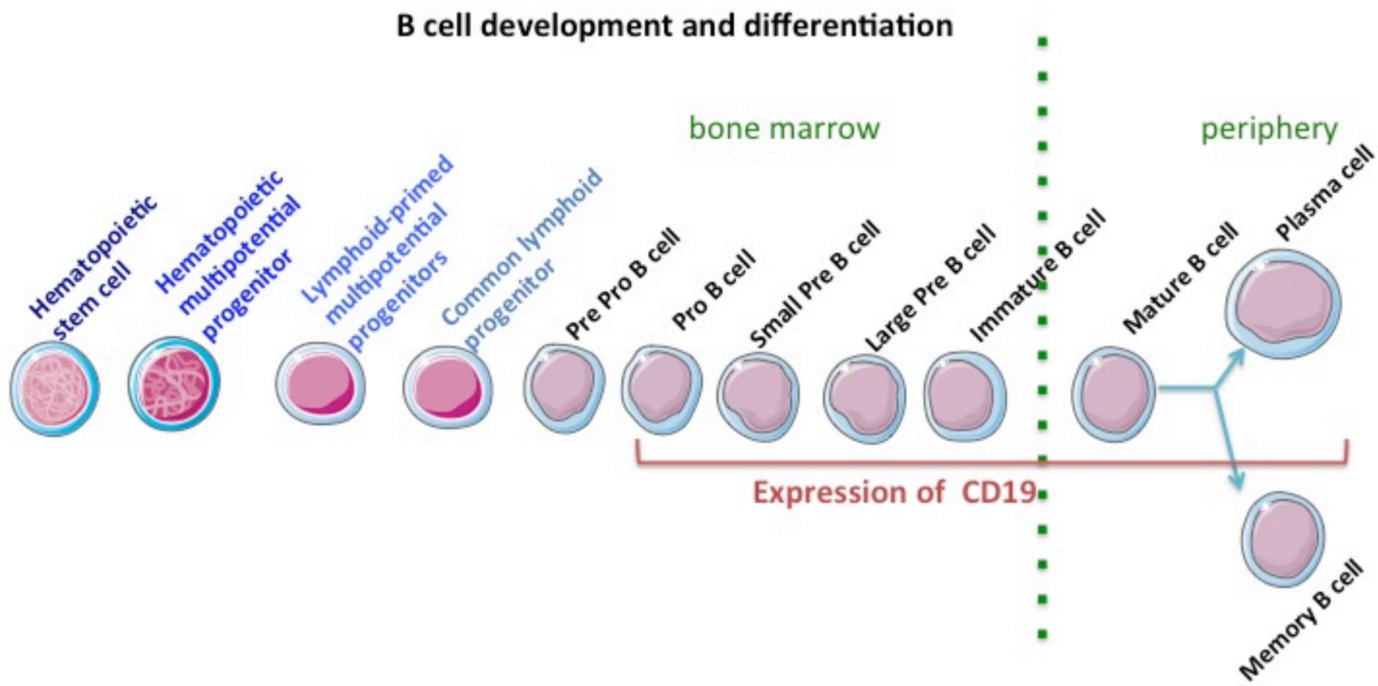

B cell development and differentiation
bone marrow
periphery
Hematopoietic stem cell
Hematopoietic multipotential progenitor
Lymphoid-primed multipotential progenitors
Common lymphoid progenitor
Pre Pro B cell
Pro B cell
Small Pre B cell
Large Pre B cell
Immature B cell
Mature B cell
Plasma cell
Memory B cell
Expression of CD19

FIGURE 1

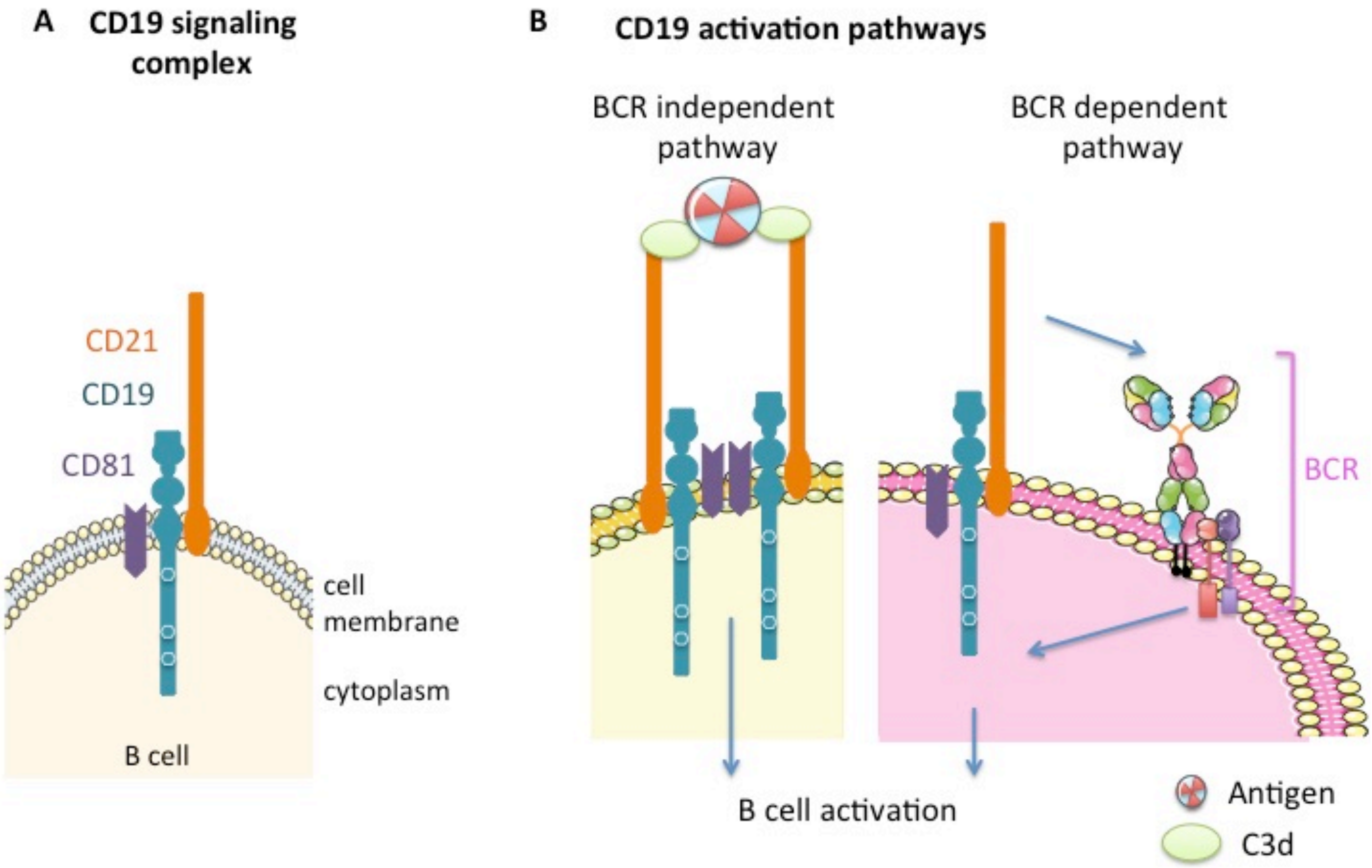

A CD19 signaling complex
CD21
CD19
CD81
cell membrane
cytoplasm
B cell
B CD19 activation pathways
BCR independent pathway
BCR dependent pathway
BCR
B cell activation
Antigen
C3d

FIGURE 2

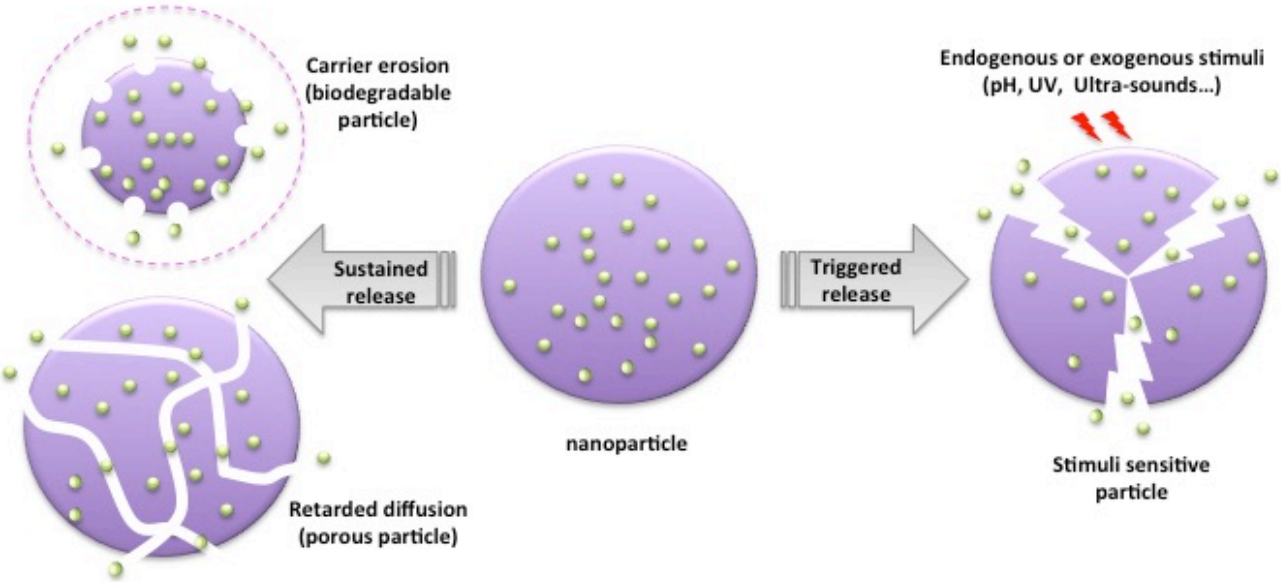
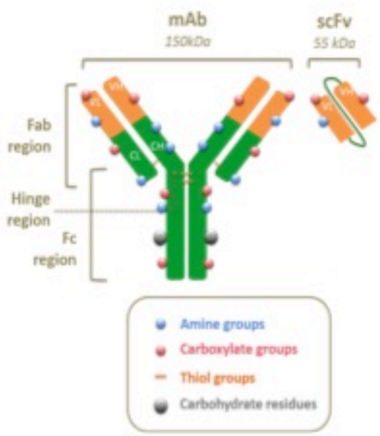

Carrier erosion
(biodegradable
particle)
Endogenous or exogenous stimuli
(pH, UV, Ultra-sounds…)
Sustained
release
Triggered
release
nanoparticle
Stimuli sensitive
particle
Retarded diffusion
(porous particle)

FIGURE 3

A



B

Name	Size	Pharmacokinetics	Valency/ specificity	Strenghs & Weaknesses
mAb	~ 150 kDa *>150 kDa, depending on its glycosylation profile*	long systemic clearance *half-time ~ few days to weeks*	Monospecific *bivalent*	High specificity/sensitivity towards their target Costly to produce Highly immunogenic
FAb	55 kDa	short systemic clearance *half-time ~ 10 h*	Monospecific *monovalent*	Rapid tumor targeting Less immunogenic than mAb Improved tumor penetration compared to mAb Low avidity (monovalency) Renal toxicity (high renal uptake)
$F(ab)_2$	110 kDa	short systemic clearance *half-time ~ 10 h*	Bispecific *bivalent*	Approved by FDA Better avidity for the target than mAb Renal toxicity (high renal uptake)
$F(ab)_3$	165 kDa	very short systemic clearance *half-time ~ 4-5 h*	Trispecific *trivalent*	High avidity for the target (multivalency) Improved tumor penetration compared to the mAb Renal toxicity (high uptake)
scFv	28 kDa	ultra-short systemic clearance *half-time > 1 h*	Monospecific *monovalent*	Improved tumor penetration compared to mAb Less immunogenic than full mAb Low functional avidity (monovalency) Renal and hepatic toxicity (high uptake)
Minibody	75-105 kDa	intermediate systemic clearance	Monospecific *bivalent*	Faster tumor addressing Better therapeutic efficacy than mAb High tumor uptake Renal toxicity (high uptake)
Multimers of scFv *Diabody* *Triabody* *Tetrabody*	 50 kDa 75 kDa 100 kDa	intermediate systemic clearance	Monospecific *bivalent* *trivalent* *tetravalent*	High avidity for the target (multivalency) Higher tumor uptake than mAb Poor tumor penetration Renal toxicity (high uptake)

FIGURE 4

Anti-CD19 CAR second generation

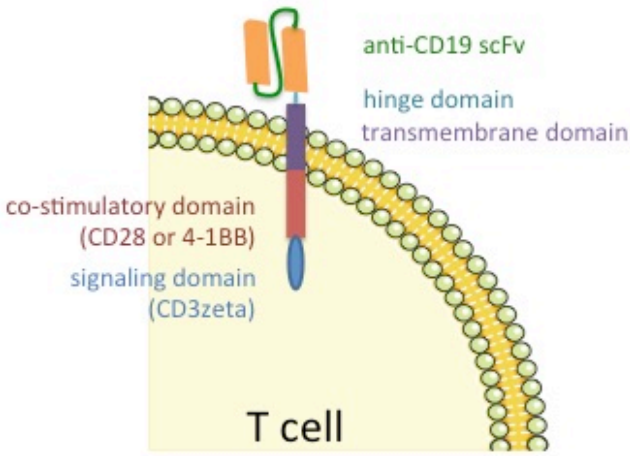
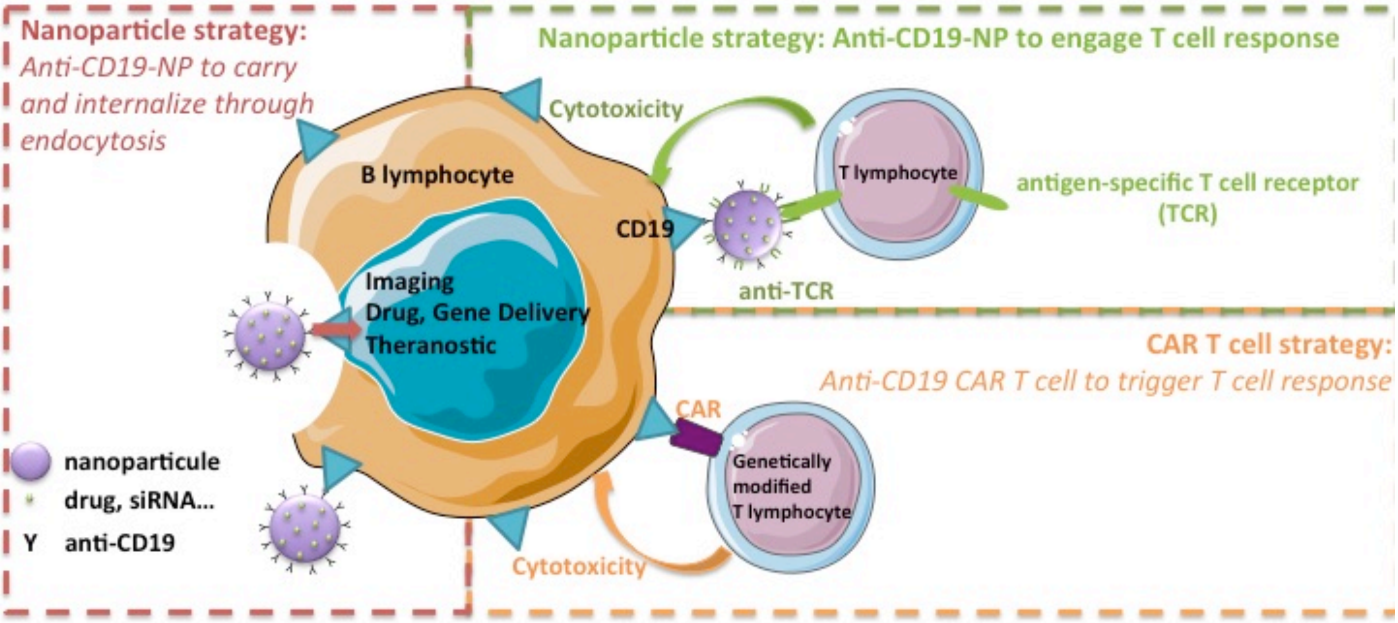


FIGURE 5


Nanoparticle strategy:
Anti-CD19-NP to carry and internalize through endocytosis
Nanoparticle strategy: Anti-CD19-NP to engage T cell response
Cytotoxicity
B lymphocyte
T lymphocyte
antigen-specific T cell receptor (TCR)
CD19
Imaging
Drug, Gene Delivery
Theranostic
anti-TCR
CAR T cell strategy:
Anti-CD19 CAR T cell to trigger T cell response
CAR
Genetically modified T lymphocyte
nanoparticule
drug, siRNA...
Y anti-CD19
Cytotoxicity