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

5
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21

22

23 **ABSTRACT**

24

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

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42 **KEY WORDS**

43

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

45

46 OUTLINES

47	1 CD19, A B CELL RESTRICTED SURFACE PROTEIN AND A RELIABLE MARKER OF B CELL	
48	MALIGNANCIES	5
49	1.1 THE FUNCTIONS OF B LYMPHOCYTES	6
50	1.2 B CELL MALIGNANCIES	6
51	1.3 THE SURFACE PROTEIN CD19: A VALIDATED TARGET PROTEIN FOR THERAPY	7
52	1.3.1 CD19 structure and function	7
53	1.3.2 Internalization of CD19 after binding to anti-CD19 antibody	8
54	1.3.3 Cells that express CD19	8
55	1.3.4 CD19 as a target for therapy	9
56	2 NANOMEDICINE IN THE TOPIC OF B CELL MALIGNANCY	10
57	2.1 NON TARGETING NANOPARTICLES FOR THERAPY AND IMAGING OF B CELL MALIGNANCY	10
58	2.2 CD19-TARGETING NANOPARTICLES	12
59	2.2.1 Imaging with anti-CD19 nanoparticles	13
60	2.2.2 Therapy with anti-CD19 nanoparticles	14
61	3 CD19-TARGETED CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS IMMUNOTHERAPY	17
62	3.1 IMMUNOTHERAPIES: ANTIBODY-BASED AND ADOPTIVE CELLULAR THERAPIES	18
63	3.1.1 The concept of CAR T cell: retargeting a cytolytic immune cell by genetic-modification to	
64	eliminate a tumor cell	18
65	3.1.2 The main biological challenges for an effective antibody-based adoptive cellular therapy	18
66	3.1.3 The choice of a relevant target antigen: CD19 gene therapy	19
67	3.1.4 The role of CAR: conferring T cell the ability to persist and expand <i>in vivo</i> and to exert cytolytic	
68	activity	19
69	3.2 CURRENT CLINICAL OUTCOMES, BENEFITS AND LIMITATIONS OF CD19 CAR T THERAPY	21
70	3.2.1 Clinical outcomes	21
71	3.2.2 Advantages	21
72	3.2.3 Limitations	22
73	4 PERSPECTIVES: HOW NANOPARTICLES AND CAR T CELL THERAPY COULD BE	
74	COMPLEMENTARY?	26
75	4.1 MULTIMODALITY	26
76	4.2 TO TRACK MALIGNANT AND CAR T CELLS	27
77	4.3 TO IMPROVE TUMOR ACCESSIBILITY	28
78	4.4 TO MANAGE TOXICITIES OF CAR T CELLS AND MODULATE THE EXPANSION OF CAR T CELLS	30
79	5 CONCLUSION	30
80		

81 **INTRODUCTION**

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

96

97 The chimeric antigen receptor (CAR) T cell therapy is a revolutionary approach of
98 targeted immunotherapy to treat cancer. In CAR T cell therapy, the therapeutic
99 effector is a genetically modified cell. CAR T cell therapy may not yet be poised to
100 overtake chemotherapy as the standard of care, however, it is looking as a promising
101 treatment for certain patients with no other feasible therapeutic option, such as in
102 relapsed or refractory leukemia. An alternative research approach for the treatment
103 of cancer is offered by nanoparticles, which have been proposed as carriers for drug
104 encapsulation in the 60's. Since then, a variety of organic and inorganic
105 nanoparticles, with sizes ranging from *circa* 5 nm to 200 nm, have been designed for

106 a wide range of applications including targeted drug delivery and imaging, thus
107 boosting the activity of nanomedicine, with some remarkable results particularly in
108 the field of cancer diagnosis and therapy.

109

110 In this perspective article, we will propose to discuss the challenges of nanoparticles
111 and CAR T cells in the context of hematological malignancies. We will focus on CD19
112 targeting objects: anti-CD19 nanoparticles and anti-CD19 CAR T cells because those
113 genetically modified cells are the most widely developed in clinical setting.

114 In the first part, we will introduce B cell malignancies and their CD19 surface marker,
115 then we will present the positioning of nanomedicine in the topic of B cell malignancy,
116 before exposing CAR T technology. Finally, we will discuss the complementary
117 approaches between nanoparticles and CAR T cells. From the biological point of
118 view, anti-CD19-grafted nanoparticles and anti-CD19 CAR T cells target the same B
119 cell lineage. From the therapeutic perspective, nanoparticles and CAR T cells
120 approaches share common objectives: the optimization of therapeutic effect on target
121 cells and the minimization of adverse effects. However, the mechanisms of action are
122 different (**see the graphical abstract**). It seems reasonable to conceive that
123 nanoparticles could play a significant role for the potentiation of, and the cooperation
124 with CAR T cell therapy in the future.

125

126 **1 CD19, A B CELL RESTRICTED SURFACE PROTEIN AND A RELIABLE** 127 **MARKER OF B CELL MALIGNANCIES**

128

129 1.1 THE FUNCTIONS OF B LYMPHOCYTES

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

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

147

148 1.2 B CELL MALIGNANCIES

149 B cell malignancies are hematological cancer characterized by uncontrolled
150 proliferation of B lymphocytes blocked along their differentiation process. B cell
151 malignancies are classified as leukemia (which develops in the bone marrow and
152 disseminates into the body), lymphoma (a cancer of the lymphatic system
153 characterized by the development of a cancer cells in lymph nodes) and myeloma

154 (cancer of mature B lymphocytes in the bone marrow) (review in (Wang et al., 2012)).
155 B cell malignancies represent 4% of all cancers in adults and 40% of all cancers in
156 children. The clinical outcomes of these cancers under standard chemotherapy
157 depend on the type of B cell malignancies. For instance, children with B-Acute
158 Lymphoblastic Leukemia (ALL) have an overall good prognosis, but some of them
159 are refractory to chemotherapy or develop multiple relapses and have a poor
160 prognosis (review in (Park et al., 2016)). Relapsed or refractory B cell ALL in adults
161 are associated with a poor prognosis (review in (Geyer and Brentjens, 2016)).

162

163 1.3 THE SURFACE PROTEIN CD19: A VALIDATED TARGET PROTEIN FOR THERAPY

164 1.3.1 CD19 structure and function

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

177

178 1.3.2 **Internalization of CD19 after binding to anti-CD19 antibody**

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

183

184 1.3.3 **Cells that express CD19**

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

200

201 1.3.4 CD19 as a target for therapy

202 Twenty years ago, CD19 was already proposed as a « suitable target for
203 immunotoxin-mediated treatment of aggressive forms of B cell lymphomas and
204 leukemia that responds poorly to conventional chemotherapy» (Uckun et al., 1988).
205 Currently, CD19 antibody-based therapy has become reality to treat B cells
206 malignancy. In the 2010's, various strategies harnessing the potential of targeting B
207 cells restricted to CD19 antigen were in development: antibody-drug conjugate, Fc-
208 engineered human CD19 antibody with antibody-dependent cell-mediated
209 cytotoxicity, chimeric antigen receptor, etc. (Hammer, 2012). The most advanced
210 anti-CD19 therapy is the Blinatumomab (BLINCYTO®, Amgen) (review in (Hammer,
211 2012)) (Goebeler and Bargou, 2016), a bispecific CD19-directed CD3 T cell engager
212 (BiTE) antibody construct. Blinatumomab binds specifically to CD19 expressed on
213 the surface of cells of B-lineage origin, and to CD3 expressed on the surface of T
214 cells. It brings both cells in contact so that the activated T cells can kill the B cells.
215 Blinatumomab is approved by the US Food-and-Drug-Administration (FDA) and the
216 European Commission (EC) for the treatment of Philadelphia chromosome-negative
217 relapsed or refractory B-ALL, in adults (USA and EC) as well as in children (USA
218 only). Additionally, anti-CD19 antibodies are also in development for
219 radioimmunotherapy in preclinical studies. ¹³¹I-labeled anti-CD19 antibody has been
220 largely explored for conventional ¹³¹I radioimmunotherapy because antigen rapidly
221 internalizes upon binding of antibody – resulting in catabolism and release of ¹³¹I
222 (Scheinberg and Strand, 1983). Moreover, ⁹⁰Y-particle-labeled anti-CD19 antibody
223 has shown an efficacy comparable to ⁹⁰Y-labeled anti-CD20 antibody in
224 radioimmunotherapy of mice with xenografts of human B lymphoma cell lines (Ma et
225 al., 2002).

226

227 **2 NANOMEDICINE IN THE TOPIC OF B CELL MALIGNANCY**

228

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

234

235 **2.1 NON TARGETING NANOPARTICLES FOR THERAPY AND IMAGING OF B CELL**

236 **MALIGNANCY**

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

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

265 Tumors are currently diagnosed using various imaging modalities such as
266 radiography, computed tomography (CT), positron emission tomography (PET) and
267 magnetic resonance imaging (MRI) (Salem et al., 2014)(Navarro et al., 2017).
268 However, the diagnosis of hematological malignancies can be challenging due to the
269 diversity of imaging appearances and clinical behavior of these diseases (Navarro et
270 al., 2017). Multimodal imaging approaches have been proposed to overcome these
271 limitations, since they offer the ability to image with different resolutions and over
272 different temporal and spatial scales. Cistaro *et al.* demonstrated the high potential of
273 combined PET (using ¹⁸F-fluorodeoxyglucose) and MRI (using paramagnetic contrast
274 agent) in the evaluation of pediatric patients with ALL (Cistaro et al., 2017). By their

275 work, they highlighted the real need of developing hybrid PET/MRI instruments and
276 dual contrasts agents.

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

295

296 2.2 CD19-TARGETING NANOPARTICLES

297 The efficiency of imaging and treatment can be greatly improved by targeting
298 specifically the malignant cells. As mentioned above, CD19 is currently the antigen of

299 choice used to target B cells. Recently, CD19-targeting nanoparticles were designed
300 for nanomedicine by grafting anti-CD19 antibody or its derivatives (Fab, F(ab)₂...) to
301 the nanoparticles (Figure 4). As an example, Cheng *et al.* produced liposomal
302 doxorubicin targeted *via* anti-CD19 monoclonal antibody fragments: either the single-
303 chain variable fragment (scFv), or the variable fragment (Fab), or the monoclonal
304 antibody (mAb) (Figure 4). The authors compared the efficacy of the three targeted
305 constructs and concluded that the scFv single-chain variable fragment would be
306 more suitable for development of immunotherapy for the following reasons: i) it
307 contained less foreign peptides, ii) the production was easier, and iii) the cost of
308 production was more economical thanks to the expression in bacterial systems
309 (Cheng and Allen, 2008). Typically, four types of chemical functions from the
310 antibody or its derivatives (-NH₂, -COOH, -SH, -carbohydrates) can be used for
311 covalent grafting to the nanoparticle. The use of spacers such as PEG derivatives
312 lowers the risk of antibody inactivation (Chen *et al.*, 2016; Manjappa *et al.*,
313 2011)(Nguyen *et al.*, 2010)(Hong *et al.*, 2015). Alternative strategies were also
314 proposed, as the noncovalent streptavidin/biotin conjugation (Procko *et al.*, 2014)
315 (Dong *et al.*, 2014).

316

317 2.2.1 Imaging with anti-CD19 nanoparticles

318 Few anti-CD19 grafted nanoparticles for *in vitro* imaging have been published so far.
319 Nguyen *et al.* designed pegylated SERS (Surface Enhanced Raman Scattering) gold
320 nanoparticles conjugated to human anti-CD19 antibody that showed specific *in vitro*
321 targeting towards chronic lymphocytic leukemia (CLL) (Nguyen *et al.*, 2010; Walker
322 *et al.*, 2012). The functional SERS nanoparticles were composed of a gold core onto
323 which a reporter dye was adsorbed. The signals were detected by dark-field

324 microscopy and Raman spectrometry and showed no interference with conventional
325 fluorescent stains used in histology. Ramos B cells labeling through anti-CD19
326 mediator was demonstrated by Dong *et al.* by grafting an anti-CD19 antibody onto
327 Ag@SiO₂ core-shell nanoparticles (Dong et al., 2014). In this study, the authors
328 monitored the metal-enhanced fluorescence of a reporter (rhodamine B) adsorbed on
329 the surface of the nanoparticles. However, to the best of our knowledge, *in vivo*
330 imaging using anti-CD19-grafted-nanoparticles has not been reported yet.

331

332 2.2.2 Therapy with anti-CD19 nanoparticles

333 2.2.2.1 Chemotherapy: drug delivery

334 Nanoparticles decorated with anti-CD19 have already been reported as effective
335 carriers for drug delivery on *in vitro* models and preclinical studies (Table 2).
336 Doxorubicin, an inhibitor of topoisomerase involved in DNA synthesis, is frequently
337 the drug of choice for proof-of-concept, as the cytotoxic effect of this drug is well
338 demonstrated on B cells. A doxorubicin loaded immunoliposome targeting B
339 lymphocytes showed a 6-fold more cytotoxic *in vitro* activity on B cells than non-
340 targeted liposomes (Lopes de Menezes et al., 1998). Similar results were observed
341 *in vivo* with an improved survival of mice injected with anti-CD19-doxorubicin-
342 liposomes compared to non-targeted liposomes or free doxorubicin treatments
343 (Lopes de Menezes et al., 1998). Doxorubicin was also encapsulated into block-
344 copolymer nanoparticles grafted with anti-CD19. A clathrin-dependent internalization
345 pathway was identified, suggesting that the physiological internalization pathway of
346 CD19 was conserved. In comparison to the administration of free doxorubicin, both
347 improved *in vitro* apoptosis of CD19 positive cells and better survival of treated mice
348 were demonstrated (Krishnan et al., 2015). *In vivo*, mice xenografted with B cells and

349 exposed to anti-CD19-liposomes containing doxorubicin or vincristine demonstrated
350 a higher cell cytotoxicity and showed a longer survival time than mice exposed to free
351 drug (Sapra and Allen, 2004). Those anti-CD19-liposomes showed *in vitro* a greater
352 binding, a more effective internalization and an equivalent cytotoxicity on B cells
353 compared to anti-CD20-liposomes (Sapra and Allen, 2004).

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

368

369 In addition, multifunctional immunoliposomes grafted with several antibodies were
370 shown to exhibit higher selectivity, greater binding affinity, and enhanced apoptosis
371 induction of B-CLL cells (Woyach *et al.*, 2014). Yu *et al.* also proposed a dual ligand
372 conjugation on immunoliposomes (Yu *et al.*, 2013). The authors first evaluated the
373 level of expression of CD19, CD20 and CD37 antigens in several B cell lines and

374 primary B-CLL cells, and found comparable level for CD19 and CD37. They also
375 calculated the internalization rate of the three antibodies in lymphoma cells (Raji
376 cells) and confirmed the choice of anti-CD37 as the primary ligand for specific
377 targeting of B cells. Then they measured the binding efficacy of single or mixtures of
378 anti-CD19, anti-CD20 and anti-CD37 on B-CLL cells isolated from patients. Greater
379 binding efficacies occurred with dual combinations of anti-CD19 and anti-CD20, with
380 anti-CD37 antibody. The antibody ratio was finally optimized to improve this
381 synergetic effect.

382

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

393

394 **2.2.2.2 Nanoparticle-based immunotherapy**

395 An innovative strategy consists in using nanoparticles exposing antibodies in order to
396 stimulate the production of lymphocytes, or even to bridge malignant cells to killer T
397 cells (**see the graphical abstract**). Schütz *et al.* designed nanoparticles termed
398 antigen-specific T cells redirectors (ATR). The ATR nanoparticles were conjugated to

399 two antibodies, an anti-TCR and an anti-CD19. The ATR nanoparticles provided a
400 physical proximity between T cells and tumor cells, and redirected T cells to kill tumor
401 cells (Schütz et al., 2016). *In vivo* assays on mice xenografted with lymphoma cells
402 and injected with ATR nanoparticles showed smaller tumors and an improved
403 survival compared to control mice.

404

405 **3 CD19-TARGETED CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS**

406 **IMMUNOTHERAPY**

407

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

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

421

422 3.1 **IMMUNOTHERAPIES: ANTIBODY-BASED AND ADOPTIVE CELLULAR THERAPIES**

423 3.1.1 **The concept of CAR T cell: retargeting a cytolytic immune cell by genetic-**
424 **modification to eliminate a tumor cell**

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

435

436 3.1.2 **The main biological challenges for an effective antibody-based adoptive cellular**
437 **therapy**

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

445 Adoptive T cell therapy with chimeric antigen receptor engineered T cells (CAR T

446 cells) has shown substantial clinical results against B cell malignancies (Porter et al.,
447 2011; Kochenderfer et al., 2012 ; Brentjens et al., 2013). The fact that CAR T cell
448 therapy approach has proven to be of some effectiveness across a range of
449 hematological malignancies (Gill and June, 2015) may be partly explained by the
450 choice of a relevant target antigen (for instance CD19) and by the fact that those
451 malignancies reside in the natural sites that adoptively transferred T cells naturally
452 invade (review in (Newick et al., 2016)).

453

454 3.1.3 The choice of a relevant target antigen: CD19 gene therapy

455 As mentioned previously, CD19 is a reliable target antigen for antibody-based
456 therapy (review in (Hammer, 2012)(Li et al., 2017)). More than half of all CAR-
457 modified T cell studies in hematological malignancies have targeted CD19 antigen
458 (review in (Beatty and O'Hara, 2016)). CD19-specific CAR T cells have demonstrated
459 potent activity in B cell ALL and lymphomas including CLL and non-Hodgkin
460 lymphoma (Porter et al., 2011 ; Grupp et al., 2013; Maude et al., 2014 ; Davila et al.,
461 2014 ; Lee et al., 2015 ; Brudno et al., 2016 ; review in Beatty and O'Hara, 2016).

462

463 3.1.4 The role of CAR: conferring T cell the ability to persist and expand *in vivo* and to 464 exert cytolytic activity

465 3.1.4.1 Design of CAR

466 The chimeric antigen receptor (CAR) is composed by two main modules: (i) an
467 extracellular component that recognizes a cell surface protein (e.g. CD19) (this
468 extracellular moiety is a single-chain variable fragment (scFv) derived from an
469 antibody) linked to (ii) an intracellular component consisting in T cell signaling

470 domains of the T cell receptor (e.g. CD3 ζ) including co-stimulatory domains (e.g.
471 CD28, or 4-1BB) involved in T cell activation (**Figure 5**) (review in (Beatty and
472 O'Hara, 2016) and (Geyer and Brentjens, 2016)). The extracellular component is
473 responsible for redirecting T cell specifically to the human tumor antigen whereas the
474 intracellular component sustains T cell activation, supporting cell expansion and
475 cytokine release resulting in cytolytic activity.

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

483

484 3.1.4.2 *Mechanism of action of CAR T cells*

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

494

495 3.2 **CURRENT CLINICAL OUTCOMES, BENEFITS AND LIMITATIONS OF CD19 CAR T**

496 **THERAPY**

497

498 3.2.1 **Clinical outcomes**

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

509

510 3.2.2 **Advantages**

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

518

519 3.2.3 Limitations

520 3.2.3.1 Genetic modification of autologous T cells

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

534

535 3.2.3.2 The need of lymphodepletion for the patient

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

542 of lymphodepletion prior to infusion of CAR T cells continues to be often incorporated
543 in clinical trials using CAR T cells.

544

545 3.2.3.3 *Toxicity for the patient*

546 The medical community will have to overcome clinical challenges related to CD19-
547 targeted CAR T cells (Geyer and Brentjens, 2016; Park et al., 2016). Major side-
548 effects, particularly cytokine release syndrome, neurological toxicities, and B cell
549 aplasia have been reported in all clinical trials using CD19-targeted CAR T cells. The
550 cytokine release syndrome is a severe inflammatory response syndrome that
551 appears within the hours to days following CAR T cell infusion. Clinical features
552 include fevers, muscle pain, malaise, and, in more severe cases, hypoxia,
553 hypotension, and occasionally renal dysfunction and coagulopathy. The cytokine
554 release syndrome is characterized by elevation of pro-inflammatory cytokines (e.g.
555 IL-6) and T cell activation and expansion. Tumor burden is positively correlated with
556 the risks of severe cytokine release syndrome and neurotoxicity (Brentjens et al.,
557 2013) (Turtle et al., 2016). The cytokine release syndrome can be life-threatening
558 and requires intensive supportive care. Mitigating strategies to reduce cytokine
559 release syndrome frequency and severity comprise anti-IL-6 receptor antibody,
560 steroids, and possibly a protocol-specified algorithm to potentially start pre-emptive
561 treatments (Maude et al., 2014; Lee et al., 2014 ;Turtle et al., 2016; Ruella et al.,
562 2017).

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

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

567 Limiting B cell aplasia for CD19-targeted CAR T cells has been successfully
568 managed with intravenous immunoglobulin replacement therapy (Frey and Porter,
569 2016). Novel approaches to limit B cell aplasia are under investigation as the use of
570 antigen-specific inhibitory CAR to protect normal B cells (Fedorov et al., 2013).

571

572 3.2.3.4 *CD19-antigen escape*

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

583

584 3.2.3.5 *Infused dose, composition, and control of expansion and function of CAR T cells*

585 So far, the different clinical trials have not led to the identification of a clear
586 correlation between higher CAR T cell infused dose and greater efficacy or CAR T
587 cell persistence (Porter et al., 2011 ; Grupp et al., 2013; Maude et al., 2014 ; Davila
588 et al., 2014 ; Lee et al., 2015 ; Brudno et al., 2016) (review in (Park et al., 2016;
589 Geyer and Brentjens, 2016)). Importantly, the efficacy of CAR T cells relies on their
590 activation in response to CD19 antigen and expansion *in vivo*, making the magnitude
591 of their reactivity unpredictable (Grupp et al., 2013). For instance, anti-CD19 CAR T

592 cells have been shown to proliferate in excess of 100,000-fold in some patients,
593 ultimately accounting for over 50% of circulating lymphocytes. The lack of control
594 over CAR T cells activation and expansion *in vivo* is a limit to predict the therapeutic
595 response.

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

617 antibody-based molecular switches that act as a bridge between the target cell and
618 CAR T cells (Rodgers et al., 2016).

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

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

625

626

627 **4 PERSPECTIVES: HOW NANOPARTICLES AND CAR T CELL THERAPY**

628 **COULD BE COMPLEMENTARY?**

629

630 **4.1 MULTIMODALITY**

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

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

643

644 4.2 TO TRACK MALIGNANT AND CAR T CELLS

645 Since the proof-of-concept of CAR T cells has been validated, current developments
646 include the control of cell expansion or avoidance of CD19 escape. There is a need
647 for noninvasive tracking of the transfused T cells in patients to determine their
648 biodistribution, viability, and functionality (review in (Liu and Li, 2014)). Several
649 strategies based on nanoparticle contrast agents have been proposed using either ex
650 vivo preloaded nanoparticles on CAR T cells, or *in vivo* administration of
651 nanoparticles after CAR T cell infusion. For instance, in mouse model, CAR T
652 biodistribution has been monitored through radiolabeled-nanoparticles or contrast-
653 agent-nanoparticles loaded into CAR T cells prior to cell infusion (Bhatnagar et al.,
654 2013;Bhatnagar et al., 2014).

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

662

663 4.3 TO IMPROVE TUMOR ACCESSIBILITY

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

680 The combination of the specific properties of CAR T cells and nanoparticles seems
681 promising to enhance the efficacy of treatments. Indeed, CAR T cells will guarantee
682 longer circulation time in the blood stream and specific recognition of B cells,
683 whereas nanoparticles can bring advantageous features such as degradation of the
684 extracellular matrix, disruption of cell-cell interactions, or thermal stimulation. An
685 advance in this direction was already reported in the literature. In mouse studies,
686 Kennedy *et al.* used T cell as chaperones for gold nanoparticle delivery to enhance
687 the efficacy of nanoparticle-based photothermal therapies and imaging applications

688 by increasing accumulation at tumor site (Kennedy et al., 2011). Another innovative
689 strategy, inspired by motile and invasive cells, would be the active enzymatic
690 degradation of the tumor matrix by protease that can be associated with the
691 nanotherapeutic system. For instance, iron oxide nanoparticles coated with
692 collagenase were magnetically driven through *in vitro* extracellular matrix, at a rate
693 similar to invasive cells (Kuhn et al., 2006). Other proteolytic surfaces include
694 bromelain, an enzymatic complex belonging to the papain family and extracted from
695 pineapple which contains a mixture of 9 proteases with distinct pH and enzymatic
696 activities (Parodi et al., 2014). Local heating triggered by external sources can also
697 be used to alter the tumor environment and enhance accessibility to malignant cells,
698 based on gold nanoparticles (Gormley et al., 2013; Smith et al., 2015).

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

713

714 4.4 **TO MANAGE TOXICITIES OF CAR T CELLS AND MODULATE THE EXPANSION OF**
715 **CAR T CELLS**

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

729

730

731 **5 CONCLUSION**

732 Nanomedicine and cell therapy are two fields that have grown in parallel. Yet, those
733 approaches aim ultimately at common goals, to achieve long remission and ideally
734 the cure of the patients. In this review, based on the example of developing tools to
735 target B cell malignancy (mostly anti-CD19 nano-objects and anti-CD19 CAR T

736 cells), we have discussed their specificity, limitations and potential complementarity.
737 It appears that even if CART T cell therapy has revolutionized management of
738 patients presenting poor prognosis B cell malignancy, improvements are needed,
739 especially to predict the therapeutic response, to control the intensity and persistence
740 of the treatment, to increase tumor accessibility of the therapeutic agent to leukemic
741 stem cell niches, and to visualize residual leukemic clones, and thus prevent
742 relapses. Therefore, therapeutic developments could benefit from nanoparticles
743 advantages -mainly their multimodality combining imaging and loading capacity, their
744 tendency to accumulate at tumor sites for solid tumors and their relative easiness to
745 be produced- to fill those requirements.

746

747 **TABLES**

748

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

754

755

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

756

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

759 Abbreviation: Ag Silver; Au: Gold; Chol: Choline; DOTAP: 1,2-dioleoyl-3-trimethylammoniumpropane;
 760 DOPE: dioleoylphosphatidylethanolamine; DSPE: Distearoylphosphatidylethanolamine; EggPC: Egg
 761 yolk phosphatidylcholine; HD37-CCH: Hybridomas HD37-c-myc-Cys-His5 scFv; HSPC: hydrogenated
 762 soy phosphatidylcholine; LNP: liposomal nanoparticle ; MHC-Ig: Major Histocompatibility Complex-
 763 Immunoglobulin; NHS: N-hydroxysuccinimide; PEG: Polyethylene glycol; PLGA: poly(lactic-co-glycolic
 764 acid); SERS: Surface Enhanced Raman Scattering; SiO₂: Silicon dioxide ; SYK: Spleen Tyrosine
 765 Kinase; TCR: T cell receptor
 766

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

767
768

769 **FIGURE LEGENDS**

770

771 **Figure 1: B cell development and differentiation**

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

776

777 **Figure 2: CD19 signaling complex and activation pathways**

778 (A) Schematic representation of the CD19 signaling complex. The CD19 complex is
779 composed of CD21, CD81 and CD19 transmembrane proteins. CD19 possesses an
780 intracellular tail with multiple tyrosine-kinase residues involved in signal transduction.

781 (B) The first pathway of CD19 activation is dependent on the B cell receptor (BCR): it
782 is a co-receptor for BCR signal transduction. The second pathway is independent of
783 the BCR: the CD19 complex is able to bind activated complement fragment C3d and
784 modulates BCR signaling (Figure adapted from (Wang et al., 2012)).

785

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

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

791

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

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

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

802

803 **Figure 5: Chimeric antigen receptor (CAR)**

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

807

808 **LIST OF ABBREVIATIONS**

809

810 ALL: acute lymphoblastic leukemia

811 Ag: Silver

812 Au: Gold

813 BCR: B cell receptor

814 B-ALL: B cell acute lymphoblastic leukemia

815 CAR: chimeric antigen receptor,

816 CL: Constant Light chain;

817 CH: Constant Heavy chain

818 Chol: Choline

819 CLL : chronic lymphocytic leukemia

820 DOTAP: 1,2-dioleoyl-3-trimethylammoniumpropane

821 DOPE: dioleoylphosphatidylethanolamine

822 DSPE: Distearoylphosphatidylethanolamine

823 EC : European Commission

824 EDC: (1-ethyl-3-(3- dimethyl-aminopropyl)carbodiimide hydrochloride

825 EggPC: Egg yolk phosphatidylcholine

826 EPR : Enhanced Permeation and Retention

827 Fab: variable region

828 Fc region: constant region

829 FDA : US Food-and-Drug-Administration

830 IFN γ : interferon gamma

831 IL6: interleukin 6

832 HD37-CCH: Hybridomas HD37-c-myc-Cys-His5 scFv

833 HSPC: hydrogenated soy phosphatidylcholine

834 LNP: liposomal nanoparticle

835 mAb: monoclonal antibody

836 MGITC: Malachite Green Isothiocyanate

837 MHC-Ig: Major Histocompatibility Complex-Immunoglobulin

838 MPS: mononuclear phagocyte system

839 MRI : Magnetic Resonance Imaging

840 MRI/CT : magnetic resonance imaging/ computerized tomography

841 MRI/PET : magnetic resonance imaging/ positron emission tomography

842 NHS: N-hydroxysuccinimide

843 NP:nanoparticle

844 PEG: Polyethylene glycol

845 PLGA: poly(lactic-co-glycolic acid)

846 PVP: polyvinylpyrrolidone

847 QD: quantum dots

848 RES : reticuloendothelial system

849 SERS: Surface Enhanced Raman Scattering

850 SiO₂: Silicon dioxide

851 siRNA:small interference RNA

852 SMCC: N-succinimidyl 4-(N maleimidomethyl)cyclohexane-1-carboxylate

853 SPDP: N-succinimidyl 3-(2-pyridylthio)propionate

854 SPECT: single, photon emission computed tomography

855 scFv: single-chain variable fragment

856 SYK: Spleen Tyrosine Kinase

857 TCR: T cell receptor

858 TEM: transmission electron microscopy
859 UCNPs : up-converting nanoparticles
860 VL: Variable Light chain
861 VH: Variable Heavy chain
862

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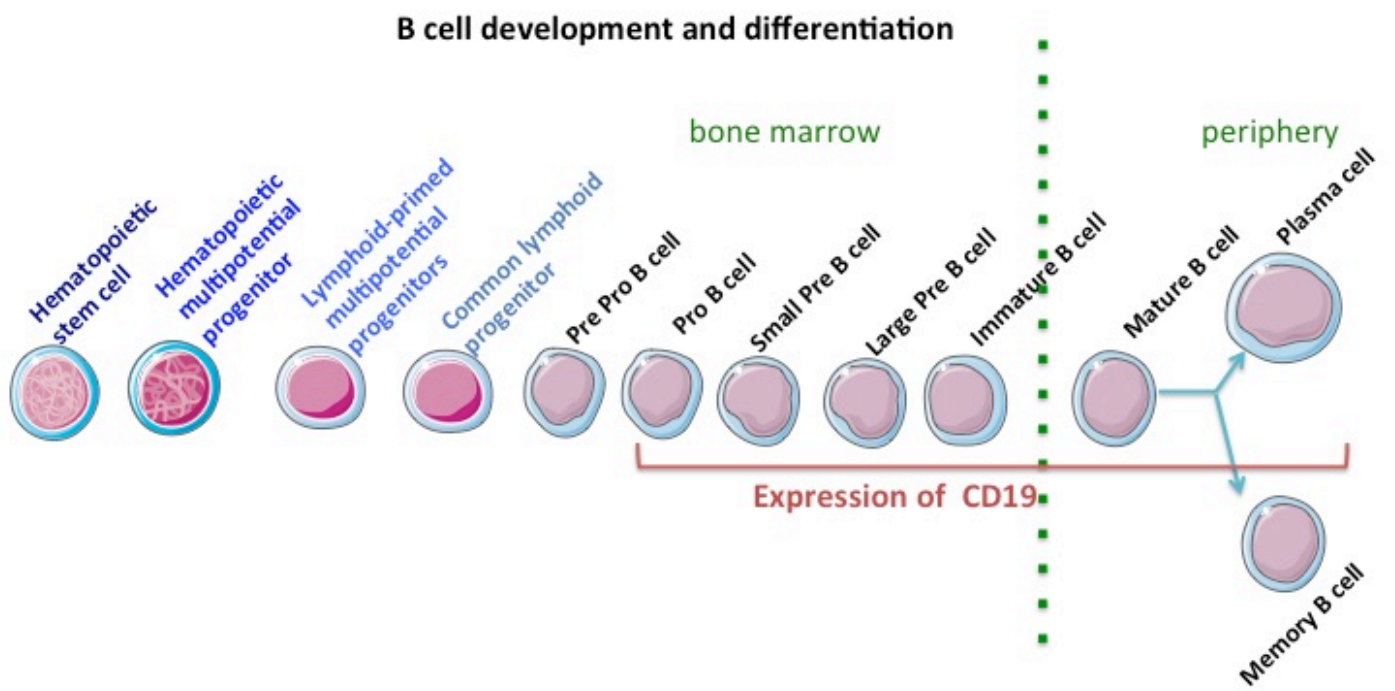
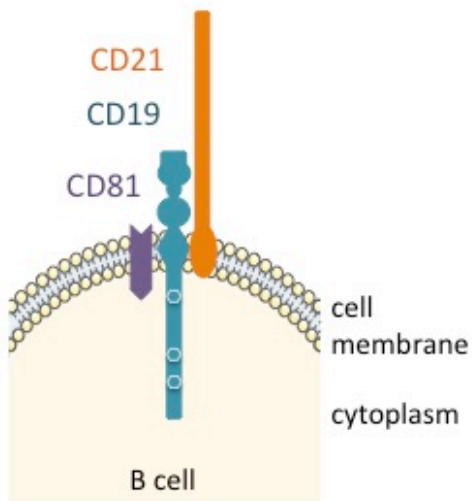


FIGURE 1

A CD19 signaling complex



B CD19 activation pathways

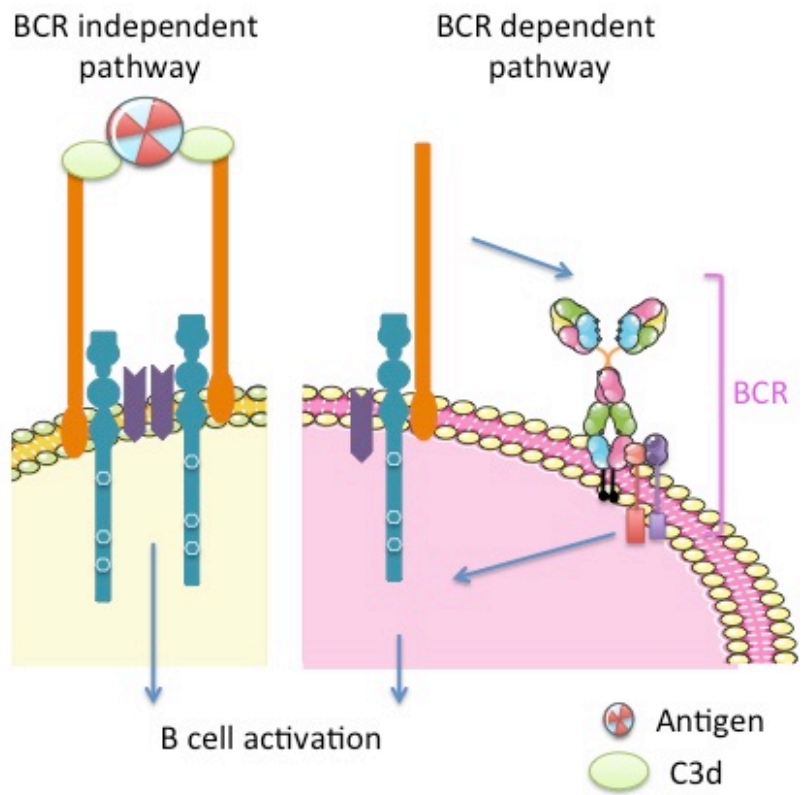


FIGURE 2

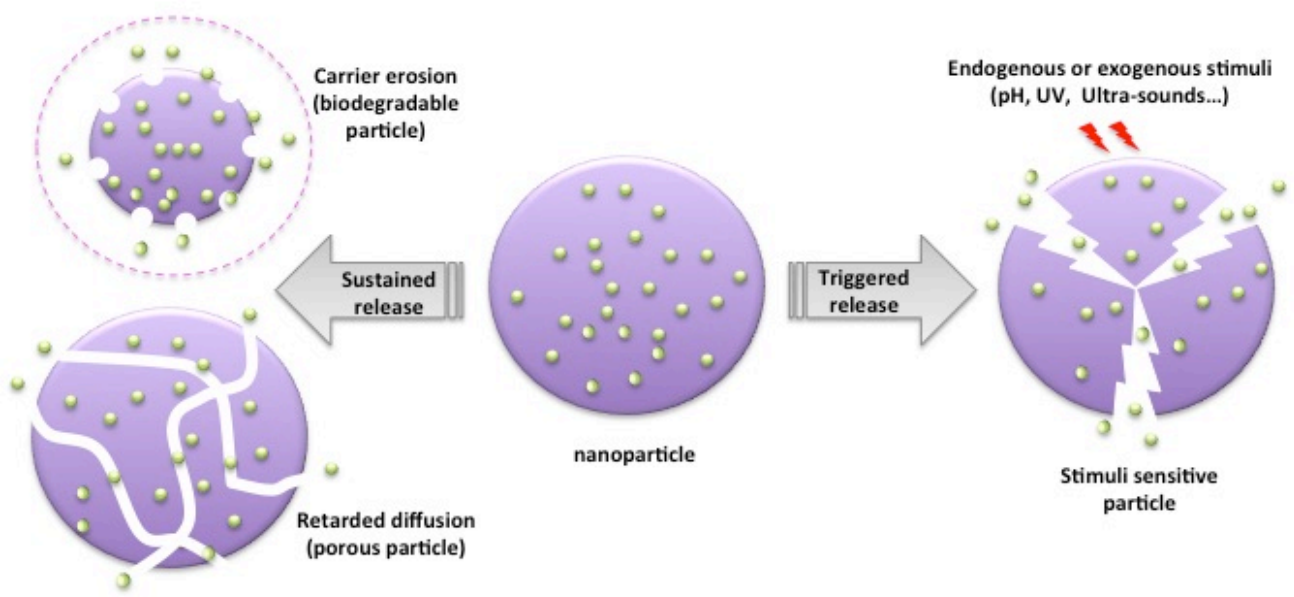
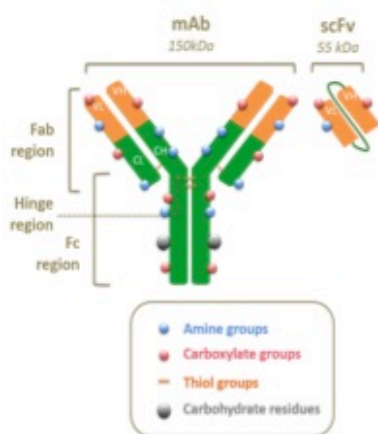


FIGURE 3

A



B

Name	Size	Pharmacokinetics	Valency/ specificity	Strengths & Weaknesses
mAb	~ 150 kDa	long systemic clearance <i>half-time</i> – few days to weeks	Monospecific <i>bivalent</i>	High specificity/sensitivity towards their target Costly to produce Highly immunogenic
Fab	55 kDa	short systemic clearance <i>half-time</i> ~ 10 h	Monospecific <i>monovalent</i>	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 <i>half-time</i> ~ 10 h	Bispecific <i>bivalent</i>	Approved by FDA Better avidity for the target than mAb Renal toxicity (high renal uptake)
F(ab)₃	165 kDa	very short systemic clearance <i>half-time</i> ~ 4-5 h	Trispecific <i>trivalent</i>	High avidity for the target (multivalency) Improved tumor penetration compared to the mAb Renal toxicity (high uptake)
scFv	28 kDa	ultra-short systemic clearance <i>half-time</i> > 1 h	Monospecific <i>monovalent</i>	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 <i>bivalent</i>	Faster tumor addressing Better therapeutic efficacy than mAb High tumor uptake Renal toxicity (high uptake)
Multimers of scFv			Monospecific	High avidity for the target (multivalency)
Dibody	50 kDa	intermediate systemic clearance	<i>bivalent</i>	Higher tumor uptake than mAb
Tribody	75 kDa		<i>trivalent</i>	Poor tumor penetration
Tetobody	100 kDa		<i>tetavalent</i>	Renal toxicity (high uptake)

FIGURE 4

Anti-CD19 CAR second generation

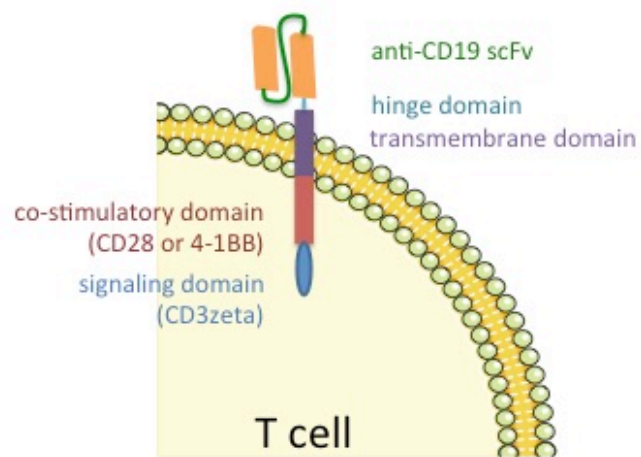


FIGURE 5

