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## Immunotherapy perspectives in the new era of B-cell editing

*Running title: Engineered B cells for therapy?*

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### **Abstract:**

Since the early days of vaccination, targeted immunotherapy has gone through multiple conceptual changes and challenges. It now provides the most efficient and up-to-date strategies for either preventing or treating infections and cancer. Its most recent and successful weapons are autologous T cells carrying chimeric antigen receptors, engineered purposely for binding cancer-specific antigens and therefore used for so-called adoptive immunotherapy. We now face the merger of such achievements in cell therapy: using lymphocytes redirected on purpose to bind specific antigens and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) revolution, which conferred genome-editing methodologies with both safety and efficacy. This unique affiliation will soon and considerably expand the scope of diseases susceptible to adoptive immunotherapy and of immune cells available for being reshaped as therapeutic tools, including B cells. Following the monumental success story of passive immunotherapy with monoclonal antibodies (mAbs), we are thus entering into a new era, where a combination of gene therapy/cell therapy will enable reprogramming of the patient's immune system and notably endow his B cells with the ability to produce therapeutic mAbs on their own.

## 29 1. Introduction

30  
31 Various strategies are currently available for passive immunotherapy, notably with monoclonal antibodies  
32 (mAbs) mimicking endogenous immunoglobulins for targeting an antigen (Ag), and thus well tolerated.  
33 Active immunotherapy is, on the contrary, based on the patient's own immune system, as after  
34 vaccination. Finally, adoptive immunotherapy reshapes autologous Ag-specific cells on purpose and is  
35 now to be boosted by new genetic engineering methods. Although future chimeric antigen receptors  
36 (CAR) T-cell protocols will likely replace lentiviral expression by clustered regularly interspaced short  
37 palindromic repeats (CRISPR)-mediated retailoring of T-cell receptor (TCR) genes, gene editing could  
38 also be applied to other cell lineages, especially B cells.

39  
40 B cells provide the best suited immunoglobulin factory for producing either membrane-bound or secreted  
41 immunoglobulin in lymphocytes or plasma cells (PCs). In lymphocytes, membrane immunoglobulin  
42 provides the Ag-binding component of the B-cell receptor, which, on Ag sensing and presentation,  
43 triggers immunoglobulin class switching and affinity maturation before activated cells differentiate into  
44 immunoglobulin-secreting PCs.

45  
46 The modular architecture of immunoglobulin was synthetically remodeled under multiple formats: single-  
47 chain (sc) fragments, minibodies, bi-specific Abs, and immunotoxins. Gene engineering methodologies  
48 now make it doable to express such retailored immunoglobulin in primary B cells, the in vivo use of  
49 which might then address multiple unmet health needs. Endogenously synthesized mAbs would notably  
50 be valuable in situations needing either (1) lifelong treatment (autoimmune, inflammatory, infectious,  
51 genetic, or residual cancer diseases), (2) permanent infusion (circumventing the issues of  
52 pharmacodynamic variations seen with intravenous injection and of rapid in vivo catabolism seen with bi-  
53 and trispecific mAbs), (3) local delivery in sites where PCs are homing, and/or (4) efficient expression  
54 despite unfit structure (for mAbs affected by chemistry, manufacturing, and control issues because of  
55 nonoptimal structures).

56  
57 This review provides an overview of such recent promising advances for adoptive immunotherapy.

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59  
60

## 61 2. Immunotherapy from the origins

62  
63  
64 Active immunotherapy began centuries ago with variolation to immunize people against smallpox and led  
65 to the concept of vaccination with viruses closely related to a pathogen but attenuated or non-pathogenic  
66 (**Figure 1**). Many vaccines now consist of purified or synthetic microbial components or simply nucleic  
67 acids encoding them. Recombinant viruses also provide platforms for developing new vaccines against  
68 emerging pathogens such as the recently arisen SARS-CoV-2.

69  
70 Passive immunotherapy reached an initial milestone with a Nobel Prize in Medicine (1901), awarded to  
71 Behring for serotherapy of diphtheria, based on the administration of serum from convalescent patients.  
72 Besides infections, anti-rhesus D immunoglobulin G (IgG) from immunized donors are also widely  
73 administrated to mothers after delivery to prevent alloimmunization. Finally, passive immunotherapy  
74 strategies now include a huge array of recombinant mAbs targeting tumor or microbial Ag for specifically  
75 treating multiple disorders.

76  
77 Cytokines can also be used to modulate immune responses, and inversely, mAbs are available for  
78 counteracting the action of tumor necrosis factor  $\alpha$  or interleukin-6 (IL-6) in inflammatory conditions,  
79 notably those resulting from adoptive immunotherapy.

80  
81 Cell therapy began in the 1950s for treating leukemia with bone marrow transplantation, which became  
82 safer after the discovery of the human leukocyte antigen system. Such allogenic transplants often  
83 associate with graft-versus-host disease, which can now be controlled and used for its graft-versus-tumor  
84 effects.

85  
86 Cancer therapy can also make use of autologous tumor-infiltrating lymphocytes<sup>1</sup>, which notably proved  
87 efficient for treating melanomas. Recently, it became possible to engineer T cells expressing chimeric  
88 antigen receptors (CAR-T-cells)<sup>2</sup>, and use of immune cells generated from induced pluripotent stem cells

89 also emerged<sup>3,4</sup>. Although such therapies become a new standard, using other lineages could expand the  
90 spectrum of immunotherapies, and B cells are specifically attractive in this regard, given their capacities  
91 to produce large amounts of immunoglobulin and to support immune memory.

### 92 3. Recent developments in immunotherapy

93  
94 Although antibodies and immune cells are the most specific tools for immunotherapy, new strategies for  
95 manipulating their production are further expanding the spectrum of their applications (Figure 2).

#### 96 97 98 **A. Recent developments of mAb-therapy**

99  
100 mAbs provide the largest class of biomedicines for treating cancers, infections, and autoimmunity, and  
101 their efficacy constantly improves. Murine mAbs have been largely replaced by less immunogenic  
102 chimeric, humanized, or even entirely human mAbs. Abs can be conjugated with cytotoxic drugs and  
103 with other functional proteins for conveying them to specific targets. The improved targeting of antibody-  
104 drug conjugates translates into lower toxicity of the attached drug. Antibody-drug conjugate specificity  
105 can even be increased by making use of bispecific mAbs. Strategies for enhancing mAb stability are also  
106 available either through optimized binding to the neonatal Fc receptor (FcRn) or through conjugation with  
107 hydrophilic polymers such as polyethylene glycol. Reciprocally, a classical strategy to extend the half-life  
108 of recombinant proteins and eventually strengthen their immunomodulating properties is to fuse them  
109 with an IgG Fc domain that notably results in their recycling by the FcRn. Etanercept, a soluble tumor  
110 necrosis factor receptor (TNFR)-Fc; abatacept, a soluble cytotoxic T-lymphocyte-associated protein 4  
111 (CTLA4)-Fc; and luspatercept (activinRIIb-Fc) are such immunomodulatory fusion proteins.

112  
113 There are still some limitations with mAb therapy, such as the treatment escape or the formation of  
114 aggregates<sup>5</sup>, and we thus need next-generation strategies delivering Abs with modulated half-life, effector  
115 properties, biodistribution, and toxicity. This also includes functional Ab fragments, monovalent Fabs or  
116 bivalent F(ab')<sub>2</sub>, single-domain Abs (nanobodies), and single-chain variable fragments (ScFv).  
117 Nanobodies composed of VL, VH, or V<sub>H</sub>H (ie, the type of V domain naturally found in some sc camelid  
118 Abs) are small-size molecules that remain as specific as conventional Abs. They are highly soluble, do  
119 not aggregate, and efficiently reach poorly vascularized tissues, and, in the absence of effector domains,  
120 they mostly act as antagonists or allosteric inhibitors<sup>6</sup>.

121  
122 ScFvs are composed of linked VH and VL domains, eventually combined as dimers (diabody), trimers  
123 (tribody), or even tetramers (tetrabody), to increase their avidity for the target. Associating different  
124 ScFvs can cumulate their specificities, as for bispecific T-cell engagers (BiTEs) aimed at bridging target  
125 with effector cells. Blinatumomab, for example, bridges CD19<sup>+</sup> target cells with cytotoxic T cells, and a  
126 trispecific Ab was proposed to target myeloma cells together with CD3 on T cells and the costimulatory  
127 CD28<sup>7</sup>. Similarly, natural killer cell engagers (NKCEs) associate anti-CD16 binding NK cells, with 1  
128 (BiKE) or 2 (TriKE) other scFvs specific for cancer cells. Some TriKEs additionally bind a cytokine,  
129 enhancing NK activity<sup>8</sup>. Instead of 3 Fabs, another format of NKCEs includes an Fc domain, naturally  
130 binding CD16, together with an anti-NKG2A checkpoint inhibitor Fab further increasing NK activity by  
131 blocking the NKG2A/MHC class I inhibitory signal<sup>9</sup>. Being smaller than regular mAbs, such next-  
132 generation Abs can reach a broader biodistribution<sup>10</sup>.

133  
134 Multivalent Abs are also attractive next-generation weapons against pathogens and notably brought broad  
135 anti-HIV specificity and protection in a nonhuman primate (NHP) model<sup>11</sup>.

#### 136 137 138 **B. Current stage of cell therapy with retargeted T cells (CAR T cells)**

139  
140 CAR T cell therapy is the latest success story in cell therapy. It is based on the forced expression of a new  
141 Ag-binding receptor able to activate transduced or transfected primary T cells against a given Ag (usually  
142 a tumor Ag)<sup>12</sup>. Additional modifications of engineered T cells were also proposed to ensure local  
143 secretion by T cell of a soluble anticancer molecule, using CAR T cells as micro-pharmacies<sup>13</sup>.

144  
145 As for CAR T cells tisagenlecleucel (Kymriah; Novartis) and axicabtagene ciloleucel (Yescarta; Gilead),  
146 approved in 2017 against CD19, current CAR T cells mostly rely on retroviruses and lentiviruses.  
147 Although there is no report of oncogenic insertion in the case of CAR T cells, such vectors still carry this

148 potential risk, calling for the development of safer gene delivery strategies. Electroporation is a common  
149 mean for introducing naked DNA, RNA, or proteins into cells, and CAR T cells were indeed also  
150 obtained after simple plasmid electroporation but only with transient expression<sup>14,15</sup>. DNA insertion and  
151 stable expression with rarer oncogenic integration than with retroviruses were also obtained using  
152 transposase-based systems<sup>16,17</sup>.

153  
154 DNA mini-circles (devoid of bacterial DNA) are also efficient vectors and have yielded functional CAR  
155 T cells persisting in vivo for more than 28 days<sup>18</sup>. They, however, still carry a risk of random genomic  
156 insertion.

157  
158 Electroporation of mRNA provides an alternative to DNA and can yield expression greater than 90%,  
159 with 80% cell viability<sup>19</sup>. Although restricted to transient (<1 week) expression, this is hereby attractive in  
160 terms of safety, with no risk of oncogenic integration and obviating the need for any suicide safety system  
161 for eliminating transfected cells in case of side effects<sup>20</sup>. It, however, remains to be demonstrated whether  
162 such transient CAR expression would yield cancer remission<sup>21</sup>.

163  
164 Although simple electroporation of nucleic acids is costly in terms of cell viability, use of nanoparticles  
165 captured through endocytosis was recently used for improving CAR T-cell transfection<sup>22</sup>. This strategy  
166 could also be applied for mRNA transfection<sup>23</sup>. Altogether, CAR T-cell therapy still carries limitations  
167 related to efficiency, persistence of engineered T cells, safety, and pricing. Future protocols resolving  
168 these limitations and notably including precise genome edition will crucially help to broaden their  
169 applications.

170

171

## 172 **C. Cell therapy in the era of precise genome editing**

173

174 Although random genomic insertion carries safety issues, new genome editing tools now make it possible  
175 to induce on-purpose mutations, deletions, and insertions. These tools, especially the CRISPR/Cas9  
176 system and its variants, are entering into therapeutic applications at least based on their ex vivo use<sup>24,25</sup>.  
177 Precise genome edition thus begins to be developed for safer generation of CAR T cells by targeting the  
178 TCR loci, simultaneously disrupting endogenous TCR expression and bringing expression of a specific  
179 CAR.

180

### 181 a. Recent developments of gene editing tools

182

183 After zinc finger nucleases and transcription activator-like effector nucleases first provided DNA scissors  
184 and were efficiently used for TCR gene edition<sup>26-28</sup>, CRISPR/Cas9 has become the most efficient and  
185 versatile system for genome engineering via RNA-guided cleavage<sup>29</sup>. The CRISPR/Cas9 toolbox now  
186 uses single guide RNA molecules, combining both RNA molecules necessary for initiating a specific  
187 cleavage<sup>30</sup>, and the Cas9 nuclease delivered to cells by DNA or mRNA transfection or simply as a  
188 protein<sup>31</sup>. Variants of this system such as nickases, dead Cas9 binding DNA without cleavage, or  
189 concomitant use of Cas9 inhibitors can further improve specificity or promote precise base  
190 replacements<sup>32</sup>. Cas9 breaks can be repaired by homology-directed repair (HDR) and then promote  
191 precise integration of a template DNA flanked with adequate homology arms<sup>33,34</sup>. These technologies are  
192 in constant development and strongly expand the possibilities of manipulating DNA, with a huge  
193 diversity of potential applications.

194

### 195 b. CRISPR edition in cell therapy

196

197 CRISPR tools recently allowed to insert a CD19-specific CAR in the T-cell receptor alpha (TCRA) locus,  
198 improving both CAR expression, cytotoxicity, and persistence of functional CAR T cells in a preclinical  
199 model<sup>35</sup>. Successful TCR gene replacement was then obtained both with an adeno-associated virus  
200 (AAV), which favors HDR<sup>36</sup>, and with a simple naked DNA template<sup>37</sup>. The possibility to simultaneously  
201 target several loci with CRISPR also opens the way for multiengineered universal off-the-shelf CAR T  
202 cells (designed for tolerance by various recipients and limited graft-versus-host disease). Ren et al notably  
203 succeeded in simultaneous CRISPR inactivation of *TCR*, *B2M*, and *PDCDI* genes, generating universal  
204 CAR T cells without human leukocyte antigen class I expression and capable of bypassing the PD-1  
205 checkpoint inhibition<sup>38</sup>.

206

207 The current challenge of CRISPR engineering in human T cells is to increase the proportion of  
208 successfully transformed cells, which is currently below that reached after viral transduction. Multiple  
209 attempts to improve HDR efficiency during CRISPR edition are thus currently tested<sup>39,40</sup>. Cas9 variants  
210 with higher fidelity might also reduce off-target genomic lesions<sup>41</sup>. Such developments should more  
211 efficiently yield next-generation CAR T cells in a context where this therapy is increasingly used. Beyond  
212 the remaining challenges of mastering side effects and safety issues, it is also tempting to explore gene  
213 edition strategies for the therapeutic use of other types of human lymphoid cells and notably B cells.

#### 214 215 216 4. Future immunotherapy strategies from the immunoglobulin/B-cell side

##### 217 218 A. Gene therapy for in vivo mAb production (vectored immunotherapy)

###### 219 220 a. Vectored immunotherapy with viruses

221  
222 Although retro/lentiviruses have a known risk of oncogenesis<sup>42</sup>, AAVs currently stand as convenient  
223 vectors for gene delivery, neither integrating into the host genome nor associating with any disease, and  
224 successful therapy based on AAVs was demonstrated against hemophilia<sup>43,44</sup>. Applications to mAb  
225 delivery, termed vectored immunoprophylaxis, emerged for various infectious pathogens such as HIV<sup>45</sup>  
226 (**Figure 3**) and proved efficient in mice against *Plasmodium falciparum* and Ebola virus and in monkeys  
227 against Simian immunodeficiency virus<sup>46-48</sup>. Vectored immunoprophylaxis might also treat tumors and  
228 increased survival was notably obtained with AAV-encoded trastuzumab in mice carrying HER2<sup>+</sup>  
229 tumors<sup>49</sup>.

230  
231 The limited packaging capacity of AAVs has prompted to improve the design of cassettes coding for  
232 mAb chains. Addition of a self-processing P2A peptide allowed to encode both H and L chains using a  
233 monocistronic cassette<sup>50</sup>. Several other formats of mAb-encoding cassettes were expressed from AAVs,  
234 but the frequent anti-AAV immune response constitutes a brake for clinical applications, together with  
235 antidrug antibodies targeting the therapeutic mAb. Despite such limitations, the AAV platform remains an  
236 interesting, vectored immunotherapy option.

###### 237 238 b. Naked DNA-encoded mAbs

239  
240 Naked DNA-encoded mAbs (DMAbs) stand as another platform for mAb in vivo delivery. It is relatively  
241 safe because naked DNA is neither infectious nor immunogenic by itself. Many studies thus used DMAbs  
242 to treat infection<sup>51-57</sup> or cancer<sup>58,59</sup>. This strategy was initially limited by low expression, but delivery and  
243 expression have now been optimized<sup>60</sup>.

244  
245 Two mAb formats, either complete or restricted to the Fab, were compared by the DMAb strategy in the  
246 context of chikungunya infection, with the former providing the best immunity<sup>53</sup>. Although this strategy  
247 can rely on a single DNA fragment linking H and L chain sequences with a P2A site, simultaneous  
248 injection of several DMAbs was also shown possible<sup>51,52,54</sup>. In both influenza and HIV infection,  
249 combined DMAb yielded immunity<sup>54,52</sup>. Muthumani et al highlighted another combination strategy by  
250 simultaneously injecting a DNA vaccine encoding the CHIKV envelope and a DMAb that neutralizes the  
251 CHIKV<sup>53</sup>. Patel et al, for their part, successfully used DMAb to produce a bispecific mAb that targets 2  
252 proteins essential to *Pseudomonas aeruginosa* pathogenicity. Preliminary experiments in NHPs with  
253 DMAb targeting the Zika virus suggested that this could be translatable to humans because it raised  
254 sufficient Ab levels for controlling viral load<sup>56</sup>. DMAb targeting HIV also showed strong in vivo IgG  
255 expression in NHP<sup>52</sup>.

256  
257 Besides infections, this strategy is pertinent to oncology and demonstrated significant antitumor activity  
258 in vivo (being as effective as a conventional mAb) for controlling tumor growth<sup>58,59</sup>, increasing CD8 T-  
259 cell infiltration, and decreasing the infiltration of T regulatory cells into tumors<sup>58</sup>.

260  
261 DMAb can thus be of interest for short- and medium-term treatment of various pathologies by rapidly and  
262 efficiently supporting the production of specific mAbs<sup>55</sup>. Repeated DMAb injections are, however,  
263 needed when prolonged treatment is required. Safety issues also finally remain associated with the  
264 potential risk of oncogenic random genomic insertion.

266 c. RNA injection: a safer alternative?  
267

268 As for CAR induction, in vivo mAb production has also motivated safe RNA-based approaches (with no  
269 risk of genomic insertion, oncogenic hit, or vector immunogenicity). Using lipid nanoparticles as carriers,  
270 Pardi et al optimized the cytosolic transfer of the mRNA encoding H and L chains of an anti-HIV  
271 neutralizing mAb<sup>61</sup>. A single intravenous dose of mRNA-lipid nanoparticles (LNPs) yielded in vivo mAb  
272 expression at 170 µg/mL after 24 hours, and weekly administration made it possible to maintain a high  
273 mAb concentration. This protected mice against challenge with an HIV derivative and showed that  
274 mRNA-LNP coding for mAbs could replace mAb injection. In a recent and encouraging study, Kose et  
275 al<sup>62</sup> also showed that an mRNA encoding a chikungunya-neutralizing human mAb expressed after a  
276 single intravenous injection of mRNA-LNPs decreased the viremia of mice challenged with the virus.  
277 Besides, it has also been shown that effective RNA-based approaches can be applied to cancer  
278 immunotherapy<sup>63,64</sup>.

279 Contrary to DNA injection, this strategy requires repeated administration to maintain a stable mAb level  
280 in vivo. Its transient efficacy (as for regular mAb treatment) can thus be a limitation for long-term  
281 treatments of chronic diseases, and more trials are still needed to ensure the absence of side effects.  
282 Despite this limitation, this drug format is significantly cheaper than proteins because the production of  
283 synthetic mRNA therapeutics<sup>65</sup> does not require expensive cell culture and purification systems. This is  
284 an important aspect, given the very high cost of mAb therapy.

285  
286 **B. Genome editing for in vivo mAb production**  
287

288 Genome editing technologies have strongly expanded the possibilities of manipulating DNA. When  
289 applied to Ab production, they allow more precise control via gene engineering of B-lineage cells.  
290

291 Although in vivo cell modification therapies might be a future grail, ex vivo modification after cell  
292 sorting obviates the need for cell targeting<sup>66</sup>. Another advantage is the possibility to analyze and  
293 characterize the modified cells before reintroducing them back into the host<sup>67</sup>. In the context of mAb  
294 therapy, B-lineage cells isolated from peripheral blood or lymphoid organs are perfect targets, because  
295 they are the ultimate antibody-secreting cells<sup>68</sup>. A system where B cells are isolated from peripheral  
296 blood, modified ex vivo for mAb secretion and injected back into the organism (**Figure 4**), would be an  
297 ideal therapeutic strategy with applications for both cancer and viral infection treatment<sup>69</sup>.  
298

299 Successful B-cell modification was first obtained using lentiviral transduction methods<sup>70-72</sup>. Primary  
300 human hematopoietic cells, including B cells, were efficiently transduced with lentiviral vectors encoding  
301 anti-HIV broadly neutralizing Abs. Edited cells engrafted and persisted in blood and lymphoid tissues in  
302 vivo in humanized mice, efficiently secreting antiviral broadly neutralizing Abs<sup>72</sup>.  
303

304 However, nonspecific lentiviral insertions could target regions essential for cell viability or function,  
305 compromising clinical applications<sup>69</sup>. CRISPR/Cas9 site-specific cleavage can by contrast result in  
306 precise insertion by HDR<sup>67,69</sup>. Cas9, guide RNA (gRNA), and the repair template for HDR can be brought  
307 into target cells as plasmids, mRNAs, or a ribonucleoprotein (RNP)<sup>66,69</sup>. In primary human B cells, RNPs  
308 seem to be highly efficient, whereas Cas9-encoding DNA and mRNA often lead to poor or no DNA  
309 cleavage<sup>66,69</sup>. To optimize HDR in cells at the S/G2 phases of the cell cycle<sup>34,73,74</sup>, various B-cell expansion  
310 mixtures were tested<sup>66,69,75</sup>, and the optimal B-cell activation cocktail included CD40L, cytosine-  
311 phosphate-guanine (CpG), IL-2, IL-10, and IL-15<sup>75</sup>.  
312

313 Efficient CRISPR/Cas9-mediated knockout in human B cells was obtained in several conditions<sup>66,69,75,76</sup>.  
314 Cheong et al<sup>77</sup> used lentiviral vectors carrying Cas9 and gRNA sequence. By using 2 gRNAs, 1 specific  
315 for a region near S<sub>μ</sub> and 1 near S<sub>γ</sub>, they induced deletions and efficiently mimicked class switch  
316 recombination in both mouse and human primary B cells. They also succeeded in generating Fab' fragment-secreting hybridomas after deleting the Fc domain-coding region, with secretion at a level  
317 comparable to the original complete immunoglobulin. This strategy would simplify the process of  
318 producing Fab' in vitro as proteins, which is currently based mainly on protease cleavage. Recently, an  
319 integrase-defective lentiviral vector was also used to target precise insertion of an antibody cassette into  
320 the *GAPDH* gene and yielded efficient expression in plasma cells<sup>78</sup>.  
321  
322

323 Expressing transgenic immunoglobulins (ie, H2L2 polymers) in normal B cells in fact involves several  
324 challenges: high expression, stoichiometric expression of both chains, and, if possible, disruption of  
325 endogenous immunoglobulin genes to minimize the assembly of chimeric immunoglobulin (ie, randomly  
326 mixing transgenic and endogenous immunoglobulin chains of unpredictable specificity). It is thus  
327 desirable to design transgenic protocols also disrupting endogenous immunoglobulin production. HDR  
328 knock-in (KI) at the immunoglobulin locus was thus explored using various strategies. The first  
329 successful immunoglobulin gene KI in B cells used an RNP/AAV combination<sup>69,75,79</sup>. Reporter cassettes  
330 were efficient inserted<sup>69,75</sup>, and gene modification for secretion of a survival factor was achieved in human  
331 PCs<sup>75</sup>. Moffett et al<sup>79</sup> engineered the IgH locus to make cells secrete a mAb that bound the respiratory  
332 syncytial virus (RSV). Their cassette was introduced upstream of the E<sub>μ</sub> enhancer and included a heavy  
333 chain promoter, the light (L) chain, a long linker, and the variable region of the H chain. Such an sc  
334 strategy both disrupted endogenous IgH chain production and forced an appropriate pairing of the  
335 transgenic heavy (H) and L chain peptides. This cassette, followed by a site for splicing of the VDJ exon  
336 to the endogenous C<sub>μ</sub> gene, thus encoded a complete (although sc) antibody. Electroporation with RNP  
337 for cleavage was followed by incubation with AAV providing the KI cassette. This strategy led to  
338 efficient expression of engineered immunoglobulin in primary human B cells, later differentiating into  
339 PCs and secreting the engineered mAb.

340  
341 Combination of RNP with double-strand or single-strand (ssDNA) DNA templates was also reported. In  
342 mice, a KI cassette for an anti-RSV mAb provided as a double-strand DNA template successfully yielded  
343 anti-RSV immunity<sup>79</sup>. Transferred cells were then able to differentiate *in vivo* into both long-lived PCs  
344 and switched memory B cells. Greiner et al<sup>66</sup> inserted ssDNA templates into the H or L chain loci to  
345 engineer B cells producing either mAbs or nanobodies that aimed at neutralizing tumor necrosis factor  $\alpha$ .  
346 Hartweiger et al<sup>80</sup> also used an ssDNA template to produce mAbs against HIV-1 and showed them to be  
347 functional when produced either by engineered mouse or human B cells. Their strategy simultaneously  
348 disrupted the  $\kappa$ L chain in primary B cells using RNPs and expressed a transgenic H and L chains cassette,  
349 inserted in the first IgH intron (downstream of JH). This cassette began with a stop cassette to interrupt  
350 the transcription of the endogenous VDJ, followed by a VH promoter, a sequence encoding Igk, a P2A  
351 cleavage site, and the VDJ region of the transgenic H chain. Splicing of a KI VDJ onto the endogenous  
352 constant region (as also cited for the work of Moffett et al<sup>79</sup>) has the advantage to be compatible with  
353 eventual class-switch recombination and production of the engineered mAb under various classes.

354  
355 For all immunoglobulin gene engineering strategies presented thus far, a common issue is the low editing  
356 efficiency, especially in primary B cells. A recent paper reported a method to increase editing yield, using  
357 nanoparticles and modifications of the HDR template<sup>81</sup>. Truncated Cas9 target sequences were added at  
358 both ends of the repair template, allowing them to recruit Cas9. Using Cas9 variants coupled with nuclear  
359 localization sequences, as a shuttle bringing the template to the genomic DNA, was also used, together  
360 with poly-L-glutamic acid to stabilize RNP nanoparticles associated with HDR template. Altogether,  
361 these tricks enhanced editing efficiency in different cell types, especially when used jointly. In B cells, it  
362 improved editing efficiency by fivefold. Poly-L-glutamic acid even permitted stabilized RNPs to resist  
363 freeze-thaw cycles and lyophilization without losing efficiency.

364  
365 Despite remaining difficulties and challenges, primary B-cell editing is obviously promising and worth  
366 efforts. Modified B cells can differentiate into memory B cells and/or Ab-secreting PCs<sup>75,79</sup>, and  
367 successful differentiation of reinfused modified B cells was reported<sup>82,83</sup>. Transferred PCs could eliminate  
368 the need for periodic mAb injections<sup>69</sup>, required for some current therapies because of limited persistence  
369 in the organism<sup>72</sup>. In some patients with immune deficiency, it could replace vaccines to protect against  
370 infections<sup>69</sup>. Contrary to the fixed structure of mAbs, engineered B cells might also be eventually capable  
371 of evolving into variants, notably through class switching, when appropriate insertion template is  
372 used<sup>80,83,84</sup>. Another possible evolution of an adoptive B-cell receptor in edited B lymphocytes might be  
373 the entry into new rounds of Ag binding selection and *in vivo* affinity maturation<sup>79,84</sup>. Such a feature  
374 would be tremendously helpful for providing durable immunity against mutating antigens such as viruses  
375 or cancer cells (evolving during chronic infection or reinfection or cancer relapse). Such adoptive  
376 immunotherapy would then dynamically reformat humoral immunity on purpose, by redesigning B-cell  
377 specificity while preserving their ability to evolve along successive immune challenges.

378  
379 Among the methodologic breaks still needed before clinical applications, increasing the amount of mAb  
380 secreted by edited B cells is first. In most published models, mAb concentration rarely reached the level  
381 needed for immunity and rapidly declined<sup>79</sup>. *Ex vivo* amplification of primary B cells and conditions for  
382 their commitment into either short or long-lived survival remain ill defined. Understanding how *ex vivo*



383 amplification could preserve or strengthen a long-lived commitment will need to be mastered for optimal  
384 B-cell engineering after optimized culture. Moreover, as only mouse models were used thus far,  
385 experimentation in NHP is required before clinical applications. By better mimicking the human patterns  
386 of viral infections or tumor progression, this will notably allow to estimate the number of cells required  
387 for a protective immunity and the best conditions for their successful graft.  
388

389 Another major concern relates to the safety of gene edition in a lineage highly exposed to oncogenic  
390 transformation and off-target mutations driven by the activation-induced deaminase activity<sup>85,79,80,84,86</sup>.  
391 Cas9 off-target mutations can be reduced by using nickase variants such as D10A<sup>85</sup> and then needing 2  
392 adjacent on-target cleavage sites. This strongly reduces the risk of an off-target double-strand break.  
393 Mutant Cas9 with reduced off-target activity are also reported<sup>86</sup>. Safety issues for B-cell adoptive  
394 immunotherapy could also partly be solved by efficient schemes for clearing edited cells in case of  
395 undesired side effects. Efficient therapies are available for total B-cell or plasma cell deletion with anti-  
396 CD20 or anti-CD38 therapeutic mAbs. More specifically, suicide strategies such as inducible apoptosis  
397 by Cas12<sup>87,88</sup> were validated in T-cell adoptive immunotherapy protocols and could be applied to edited B  
398 cells.  
399

400 Overall, despite these various efficiency and safety issues, there are already strong preliminary elements  
401 showing that adoptive B-cell immunotherapy is feasible and should soon take its part in the therapeutic  
402 arsenal, solving a number of unmet needs in human health.  
403

404  
405 **Authors contribution statement:** Conceptualization, M.Cogné, N.U, M.Cahen; Writing - original draft  
406 preparation, M.Cogné, N.U, Y.D, M.Cahen; Writing - review and editing, M.Cogné, N.U, M.Cahen, C.S,  
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408

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410

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414

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620

621 **Figures**

622 **Figure 1. A timeline of the history of immunotherapy**

623 **Figure 2. New-generation mAb structures**

624 **Figure 3. In vivo production of mAb by additive gene therapy**

625 **Figure 4. A potential cycle of B cell-mediated therapies including an ex vivo genome edition step**