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Alessia Cavazza, Ayal Hendel, Rasmus O Bak, Paula Rio, Marc Güell, Duško Lainšček, Virginia Arechavala-Gomez, Ling Peng, Fatma Zehra Hapil, Joshua Harvey, et al.

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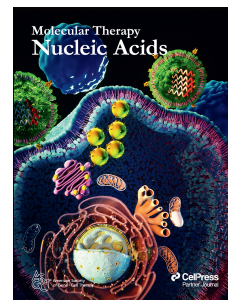
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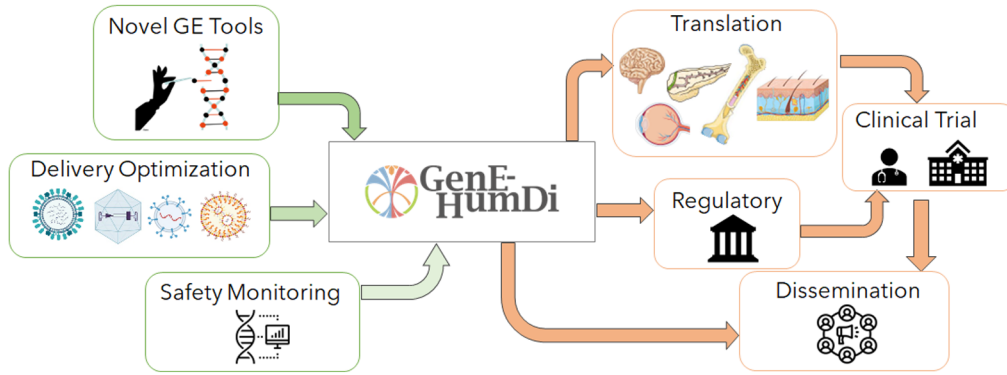
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Journal Pre-proof

1 **Progress and Harmonization of Gene Editing to Treat Human Diseases**
 2 **(Proceeding of COST Action CA21113 GenE-HumDi)**

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112 **Abstract**

113 The European Cooperation in Science and Technology (COST) is an intergovernmental
114 organization dedicated to funding and coordinating scientific and technological
115 research in Europe, fostering collaboration among researchers and institutions across
116 countries. Recently, COST Action funded the "Genome Editing to treat Human
117 Diseases" (GenE-HumDi) network, uniting various stakeholders such as pharmaceutical
118 companies, academic institutions, regulatory agencies, biotech firms, and patient
119 advocacy groups. GenE-HumDi's primary objective is to expedite the application of
120 genome editing for therapeutic purposes in treating human diseases. To achieve this
121 goal, GenE-HumDi is organized in several working groups, each focusing on specific
122 aspects. These groups aim to enhance genome editing technologies, assess delivery
123 systems, address safety concerns, promote clinical translation, and develop regulatory
124 guidelines. The network seeks to establish standard procedures and guidelines for
125 these areas to standardize scientific practices and facilitate knowledge sharing.
126 Furthermore, GenE-HumDi aims to communicate its findings to the public in accessible
127 yet rigorous language, emphasizing genome editing's potential to revolutionize the
128 treatment of many human diseases. The inaugural GenE-HumDi meeting, held in
129 Granada, Spain, in March 2023, featured presentations from experts in the field,
130 discussing recent breakthroughs in delivery methods, safety measures, clinical
131 translation, and regulatory aspects related to gene editing.

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145 **Introduction**

146 Genome editors are transformative technologies that can address genetic
147 causes of human diseases. Tremendous progress has been made across different
148 classes of genome editors, from meganucleases, zinc finger nucleases (ZFNs),
149 transcription activator-like endonucleases (TALENs), and Clustered Regularly
150 Interspaced Short Palindromic Repeats (CRISPR). The simplicity with which CRISPR
151 genome editors can be programmed by a short-associated guide RNA (gRNA) has
152 stimulated their broad application in life sciences research and clinical
153 development. Exciting additions to the CRISPR editing toolbox include base editors
154 (BE) that can precisely install certain point mutations and prime editors (PE) that can
155 copy information in a prime editing gRNA (pegRNA) into a nicked DNA target. Each
156 of these editors are now being rapidly developed into genome editing (GE)
157 medicines, by both companies and academic groups. The first GE medicine, *exa-*

158 *cel*, for treatment of sickle-cell disease (SCD) or transfusion-dependent beta-
159 thalassemia (TDT) is expected to be FDA-approved in December 2023.

160 In the context of advancing scientific and technological innovation, the COST
161 (European Cooperation in Science and Technology, cost.eu) provides a platform for
162 researchers and experts to collaborate and exchange knowledge and expertise
163 across different fields and disciplines. COST's central mission is to promote cross-
164 border collaboration and networking among researchers and institution spanning
165 various European and neighbouring countries with the aim of advancing scientific
166 and technological innovation in Europe. These initiatives involve a series of calls for
167 projects, where researchers and institutions can submit proposals for scientific and
168 technological projects related to the initiative's theme. The funding is provided to
169 selected projects, which are expected to collaborate and network within the framework
170 of the initiative. In particular, the COST Action GenE-HumDi, with 269 participants
171 distributed across 38 countries (figure 1) is focused on exploring the use of GE to
172 treat diseases that affect humans.

173 Specifically, the GenE-HumDi Action aims to promote collaboration between
174 academic institutions, pharmaceutical companies, hospitals, regulators, and patient
175 advocacy associations to accelerate the translation of GE technologies into effective
176 treatments for human diseases. During its four years of operation, the GenE-HumDi
177 network (<https://www.genehumdi.eu/>) aims to regularly discuss the establishment
178 of standard operating procedures (SOPs) and guidelines focusing on key areas for
179 the accelerated translation of GE into the clinic: 1) design of improved GE tools and
180 their delivery; 2) assessment of the safety of GE platforms; 3) creation of a roadmap

181 for the translation of GE from bench to bedside; 4) mapping of industry engagement
182 and intellectual property; and 5) evaluation and promotion of regulatory guidelines
183 for GE clinical translation and commercialization. The implementation of GenE-
184 HumDi, spanning 26 member countries of COST, is overseen by a Management
185 Committee (MC) composed of national experts, as well as a Chair, Dr Karim
186 Benbdellah, and a Vice-Chair, Dr Alessia Cavazza. The initiative has been structured
187 into seven Working Groups (WG). A dedicated WG, named "Improvement of GE
188 technologies" and led by Dr Rasmus O. Bak, has been established to consolidate
189 information on the efficiency and specificity of various existing GE tools. A "Delivery
190 Strategies" WG led by Dr Yonglun Luo has been established with the aim of
191 evaluating the optimal delivery methods of different GE tools for each cell type,
192 animal model and route of administration. The third WG, headed by Dr Ayal Hendel,
193 focuses on "safety issues" related to GE. Its main objective is to outline and
194 standardize current methodologies for assessing the cytotoxicity and genotoxicity
195 of different GE platforms, including off-target and on-target effects, unintended
196 recombination events, and cell population biases. A fourth WG, "Translation into the
197 Clinic" led by Dr Alessia Cavazza, is dedicated to the multifaceted translational
198 aspects of GE for clinical practice. The primary objective of this WG is to evaluate
199 the status of GE clinical translation in Europe for rare inherited diseases, cancers,
200 and infectious diseases, as well as to develop a roadmap for protocol adoption and
201 manufacturing of GE-based medicines. The "Technological Transfer and Industry"
202 WG headed by Monika Paule aims to draw guidelines to provide cost-effective GMP-
203 grade sourcing of GE-based medicines and promote intellectual property
204 management between collaborating partners. The WG focused on "Regulatory

205 Issues", headed by Prof Toni Cathomen, aims to ensure the regulatory adequacy of
206 preclinical models and methods used to assess the efficacy and safety of various in
207 vivo and ex vivo GE tools. This WG group also aims to promote the development of
208 regulatory guidelines for the translation of GE into clinical practice. Finally, a
209 "Dissemination" WG led by Dr Lluís Montoliu focuses on the promulgation of the
210 results stemming from our Action and the integration of research and data analysis
211 to decrease knowledge fragmentation among partners and to communicate the
212 relevant advances in this field to a wider non-scientific audience. Eighty-seven
213 scientists belonging to the Gene-HumDi Action from both academia and industry
214 from 29 different countries gathered at the Center for Genomics and Oncological
215 Research (GENyO) for the inaugural meeting of the Action (13th-15th March 2023,
216 Granada, Spain), to review the state-of-the-art of the GE field and debate on its future
217 directions. The following proceedings summarize key considerations and highlights
218 from the meeting, which revolved around the seven main pillars of the Action.

219 **Improving Gene Editing Technologies**

220 Development of GE technologies moves at an exceptional high pace. Fueled
221 by the advent of the CRISPR/Cas technology, the community has seen over the
222 past 10 years several new GE tools being developed and existing tools being
223 refined. This has shaped major research areas in (1) conventional DNA editing
224 methods relying on DNA double-strand breaks (DSBs), (2) DSB-free gene editing
225 tools such as BE and PE, (3) tools that enable site-specific integration of large
226 genetic payloads, and (4) genetic engineering tools that do not change the DNA
227 code but instead edit RNA or manipulate epigenetic and/or transcriptional status

228 of a gene. Various GE technologies have been developed and, while CRISPR/Cas-
229 based tools dominate the field, it is important to recognize alternative tools that
230 have been evaluated in clinical studies, such as ZFNs and TALENs, as well as less
231 embraced strategies for editing the genome.

232 CRISPR/Cas was originally developed to introduce site-specific DNA breaks
233 to edit genomic sequences, but as shown in **Figure 2** it has been reconfigured
234 and repurposed for a wide range of applications. Rasmus Bak (Aarhus University,
235 Aarhus, Denmark) and Julian Grünewald (Technical University of Munich, Munich,
236 Germany) presented some of the recent efforts made in developing and applying
237 new tools for genetic engineering, including BE and PE, as well as CRISPR/Cas-
238 based transcriptional modulators ^{1,2}. These tools allow researchers to modify DNA
239 in a more precise and controlled manner, with the potential to correct disease-
240 causing mutations and without the need to introduce DSBs at the target site. PE
241 can create new DNA sequences by inserting, deleting, or replacing specific
242 genomic sequences. CRISPR/Cas transcriptional modulators, on the other hand,
243 allow researchers to turn genes on or off without changing the DNA sequence ³.

244 During his presentation, Giedrius Gasiunas (CasZyme, Vilnius, Lithuania)
245 discussed the challenges associated with using the Cas9 proteins for GE
246 applications. The requirement for a protospacer adjacent motif (PAM) to bind to
247 a target, the lack of specificity, and size limitations for its viral delivery are
248 significant obstacles for Cas9-based GE. To overcome these challenges, Gasiunas
249 and his team explored the natural variation of Cas enzymes to develop RNA-
250 guided tools with diverse and potentially beneficial properties ⁴. Through
251 biochemical screens of Cas9 orthologues of the type II family and Cas12 proteins

252 from the novel type V family, the team found a wide range of activities both in vitro
253 and in cellula, making them an attractive alternative to traditional Cas9 enzymes ⁵.
254 Similarly, Lluís Montoliu (CNB-CSIC, Madrid, Spain) presented a collaborative
255 effort leading to the resurrection of some CRISPR-associated nucleases from tens
256 to thousands of millions of years ago, obtained through statistical inference
257 applying a maximum likelihood approach, while searching for novel Cas
258 nucleases from bacteria that might have not interacted with human beings and, as
259 such, had not been known by our immune system. He presented the
260 corresponding validation experiments in human cells showing that these
261 ancestral Cas nucleases were suitable for genome editing applications, with a
262 comparable efficiency to nowadays nucleases ⁶. Marc Güell (Pompeu Fabra
263 University, Barcelona, Spain) discussed the limitations of the CRISPR technology
264 when employed to generate large genomic changes and the need for improved
265 methods. To address this issue, Güell and colleagues combined the CRISPR
266 system with the gene transfer capacity of transposases to create novel gene
267 writers that can efficiently introduce large genomic changes with precise control
268 ⁷. This new system, known as Find and cut-and Transfer (FiCAT) enables
269 researchers to evolve and optimize CRISPR enzymes for a wide range of
270 applications, including gene regulation and epigenetic modifications. Dusko
271 Lainscek (National Institute of Chemistry, Slovenia) presented his work on
272 augmenting the action of CRISPR/Cas9 system by noncovalent tethering of Cas9
273 protein to the exonuclease III via coiled-coil forming heterodimeric peptides
274 (CCExo) ⁸. Indeed, bringing the exonuclease III into proximity of the Cas9-induced
275 DSB results in additional DNA recessions with final larger deletions and increased

276 rate of gene alterations. CCExo showed robust increased GE action determined
277 for several different genes in various cell lines, as well in human primary cells and
278 in somatic adult cells *in vivo*, with no additional undesired DNA cleavage
279 observed by CIRCLE-seq. As such, this system could be applied to treat different
280 diseases, which was exemplified by the speaker by using CCExo for targeting “the
281 BCR-ABL1 fusion chromosome, a main cause of chronic myelogenous
282 leukaemia, in patient’s cells and in xenograft animal model.

283 **Strategies to deliver gene editing tools**

284 Unlike the rapid and continuous development of GE technologies, approaches to
285 deliver the GE cargos specifically and efficiently into the target cells and tissues are
286 evolving at a much slower pace and now represent one of the major limiting factors
287 in advancing CRISPR therapeutic applications. Although smaller or split Cas
288 proteins were engineered to make them compatible with the low immunogenic and
289 pathogenic adeno-associated viral (AAV)⁹ vectors, concerns are not fully addressed
290 regarding the unintended off-target effects due to relatively high and long-term
291 AAV-mediated CRISPR expression, as well as CRISPR-independent adverse effects
292 associated with viral vector genome integration into host cells. Besides, it is vital to
293 select the right delivery method to achieve a better GE outcome in term of
294 efficiency, efficacy, and specificity depending on the GE strategies and applications
295 chosen.

296 The overarching goal of the “Delivery Strategies” WG3 and session is to critically
297 evaluate and identify the most effective ex vivo and in vivo delivery systems for GE
298 applications (illustrated in **Figure 3**). Specifically, the different speakers assessed the

399 suitability of various delivery methods for delivering genetic material to different cell
300 types and animal models, with the aim of advancing the field of GE and promoting
301 the development of novel therapeutic approaches, based either on viral and non-
302 viral delivery systems. As excellently illustrated by Virginia Arechavala-Gomez
303 (Biobizkaia Health Research Institute, Bilbao, Spain), delivery of nucleic acid
304 therapeutics has been the subject of much interest since early antisense
305 oligonucleotide therapies were developed with mixed results^{10,11}: while therapies
306 targeting tissues such as the eye or the central nervous system provided patients
307 with life-changing therapeutic options¹², others targeting the muscle showed
308 limited clinical benefit¹³. Researchers in the field soon realized that delivery was the
309 main hurdle stopping these new drugs from reaching their full potential¹⁴. The
310 issues related to the delivery of these compounds can be divided in two areas:
311 access to target tissue and escape from the endosomes¹⁵. Most nucleic acid
312 therapeutics, and in particular GE tools, are too large and too charged to bypass
313 these barriers on their own, hence there is an urgent need for alternative and more
314 efficient delivery systems.

315 Rosario Sanchez (GENYO-UGR, Granada, Spain) presented on the use of non-viral
316 nanoparticles as delivery systems for GE tools in cancer immunotherapy. They
317 developed a multifunctional nanodevice capable of efficient delivery of CRISPR-
318 Cas9 and GE tools to T cells, enhancing their ability to recognize and target cancer
319 cells. Additionally, the potential of polymeric and plasmonic nanoparticles as
320 delivery systems for mRNA-based therapeutics was discussed. Poly(lactic-co-
321 glycolic acid) PLGA nanoparticles offered protection and improved cellular uptake
322 of mRNA, while plasmonic nanoparticles increased mRNA concentration and uptake

323 through photothermal effects ¹⁶⁻¹⁸. The presentation also compared PLGA
324 nanoparticles and electroporation to deliver CRISPR/Cas9, highlighting their
325 advantages and limitations in terms of effectiveness, specificity, and safety. Although
326 PLGA nanoparticles showed lower editing efficiency compared to nucleofection,
327 further improvements in encapsulation methods were suggested.

328 On the other hand, dendrimers represent a special family of polymers that are
329 emerging as promising vectors for nucleic acid delivery by virtue of their well-
330 defined dendritic structure and cooperative multivalency ¹⁹⁻²⁴. Ling Peng (Aix-
331 Marseille Université, Marseille, France) discussed the potential application as well as
332 limitations of amphiphilic dendrimers to improve endosome release in nucleic acid
333 delivery. These conjugates can mimic lipid vectors and exploit membrane-fusion-
334 mediated delivery, while simultaneously retaining the multivalent properties of
335 polymer vectors that allow endocytosis-based delivery benefiting from the proton-
336 sponge effect. In the future, researchers may investigate the potential of using
337 amphiphilic dendrimers as delivery vehicles for various types of RNA therapeutics,
338 such as antisense oligonucleotides for gene silencing, small activating RNA for gene
339 activation, and mRNA and single guide RNA for GE.

340 Another emerging promising approach that is now widely utilized in clinical settings
341 for the delivery of GE reagents involves the use of synthetic lipid nanoparticles
342 (LNPs). By encapsulating specific gRNA and Cas9 mRNA, LNPs provide a DNA-free
343 CRISPR delivery system that can be readily taken up by cells through endocytosis ²⁵.
344 Julie Lund Petersen (Aarhus University, Aarhus, Denmark) discussed the formulation
345 and utilization of LNPs for the encapsulation of CRISPR RNA. Notably, their research

346 demonstrated the efficacy of LNPs in delivering GFP mRNA, as evidenced by the
347 high levels of GFP expression observed in vitro in cultured cells and in vivo following
348 subretinal and hippocampal injections in mice.

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351 **Safety Issues related to GE therapeutic applications.**

352 Despite the revolutionary nature of CRISPR/Cas9 genome editing, concerns about
353 its promiscuous nuclease activity and unintended off-target effects have been
354 raised. The off-target activity may lead to the introduction of unintended
355 insertions/deletions (indels) or structural variations, posing significant safety
356 concerns. One approach to mitigate off-target genotoxicity involves the
357 development of more specific nucleases, such as alternative Cas variants²⁶. Another
358 approach involves utilizing chemically modified gRNAs, which have been shown to
359 increase both the efficiency and specificity of the system²⁷. Lastly, extensive research
360 is dedicated to the development of sensitive assays and tools for the prediction and
361 detection of off-target sites. Strategies aimed at enhancing sensitivity include the
362 utilization of Cas9 overexpression systems, advancements in sequencing methods,
363 and the development of improved computational algorithms. The insights gained
364 from the off-target detection methods could in turn aid in the selection and
365 refinement of more precise editing systems. Apart from off-target genotoxicity,
366 additional toxicity concerns arise from the foreign DNA that may be used to deliver
367 any of the editing components, be it by non-viral or viral vectors. The presence of

368 foreign nucleic acids triggers DNA damage response (DDR) pathways, leading to
369 cell cycle arrest, transcriptional blockage, reduced proliferation, and potentially
370 apoptosis^{28,29}. Ensuring safety in the various aspects described is of utmost
371 importance, particularly as Cas9-based therapeutic GE has entered clinical trials.
372 Several talks focused on establishing a standardized protocol for the measurement
373 and evaluation of on- and off-target activity, which included generating big data to
374 develop models that better predict off-target activity. The speakers compared the
375 prediction of cell-based, cell-free, and *in-silico* off-target identification methods and
376 highlighted the need for a combination of experimental methods to assess the
377 safety of gRNAs (reviewed in **Figure 4**). Shengdar Q. Tsai (St. Jude Children's
378 Research Hospital, Memphis, USA) the keynote speaker of the meeting, discussed
379 several cell-based and biochemical methods for understanding off-target effects,
380 including integration-deficient lentiviral capture, high-throughput genome-wide
381 translocation sequencing, and genome-wide unbiased identification of DSBs
382 enabled by sequencing (GUIDE-seq), and Circularization for high-throughput
383 analysis of nucleases genome-wide effects (CHANGE-seq) among others^{30,31}. He
384 highlighted the importance of using sensitive and unbiased methods for defining
385 the potential safety and genotoxicity of editors through the lens of developing SCD
386 genome editing therapies. Ayal Hendel (Bar-Ilan University, Ramat Gan, Israel)
387 addressed the safety concerns involved in the development of an HDR-based
388 correction strategy in CD34+ hematopoietic stem and progenitor cells (HSPCs). To
389 assess potential off-target effects, the Hendel group implemented a two-step
390 approach involving the identification of off-target sites using GUIDE-seq in a Cas9
391 overexpression system, and the quantification of their activity through rhAmpSeq.

392 While high off-target activity was observed in a HEK-293 Cas9 cell line, the use of
393 ribonucleoprotein (RNP) complexes and high-fidelity (Hi-Fi) Cas9 in CD34+ HSPCs
394 significantly reduced toxicity³². Hendel also discussed viral vector-related safety
395 aspects, noting that viral HDR donors triggered a DDR proportional to the AAV
396 multiplicity of infection (MOI) used³³. Furthermore, higher viral doses led to
397 extended vector genome presence, decreased cell yields, and positive selection of
398 unedited cells. However, by reducing the viral vector dosage, significant
399 improvements were observed in HSPC survival, allowing successful T-cell
400 differentiation of corrected SCID-RAG2 patient HSPCs *in vitro*³⁴. These findings
401 highlight that minimizing the viral vector dose to strike a delicate balance between
402 non-toxicity and optimal editing efficiency, is essential for upholding the highest
403 standards of safety. Marcello Maresca (Astrazeneca, Molndal, Sweden) discussed
404 the limitations of current strategies for mapping CRISPR/Cas9 off-target effects,
405 which can impact their sensitivity. His team has developed a new off-target
406 assessment workflow using duplex sequencing, which can increase the sensitivity of
407 CRISPR/Cas9 mutation detection by one order of magnitude and the reduction of
408 false positive and negative rates, which enabled the identification of previously
409 missed off-target mutations associated with wild-type SpCas9 treatment in an *in vivo*
410 humanized PCSK9 mouse model of hypercholesterolemia. In addition to this
411 innovative technique, Maresca also discussed the development and features of a novel,
412 precise Cas9 nuclease, known as PsCas9. This new nuclease shows high intrinsic specificity
413 and is considered a promising alternative to SpCas9 for both research and clinical purposes
414 in the field of genome editing. Maresca believes that using a highly sensitive off-target assay

415 in conjunction with PsCas9 could provide more accurate genome editing treatments and
416 safety assessment.³⁵

417 Jan Gorodkin (University of Copenhagen, Copenhagen, Denmark) highlighted the
418 challenge of balancing on-target efficiency and minimizing off-target effects when
419 selecting gRNAs for a given genomic region, and emphasized the need for
420 advanced computational tools to analyze and predict gRNA activity, as well as the
421 importance of experimental validation to confirm the predicted results. In this
422 regard, Gorodkin's team developed the CRISPRon/off framework, a computational
423 methodology based on a deep learning-based predictor and a binding energy
424 model, which aims to facilitate the selection of optimal CRISPR/Cas9 gRNAs for GE
425 ³⁶⁻⁴⁰. Its web interface (<http://rth.dk/resources/crispr>) provides easy access to the
426 tools and allows users to input genomic regions of interest for gRNA design. The
427 deep learning-based predictor uses a convolutional neural network to predict on-
428 target efficiency, while the binding energy model predicts off-target effects based
429 on the binding energies of the gRNA and potential off-target ³⁶. Stefan Seemann
430 (University of Copenhagen, Copenhagen, Denmark) presented a tool called
431 CRISPRroots which combines off-target predictions, variant calling, and differential
432 expression analysis of RNA-seq data to evaluate successful on-target editing while
433 also identifying any predicted off-target effects that could be contributing to the
434 observed gene expression changes ³⁷. The tool uses a combination of off-target
435 predictions, variant calling, and differential expression analysis of RNAseq data to
436 evaluate successful on-target effects while also identifying potential off-target
437 effects in genes with altered expression. The method provides an unbiased analysis
438 of somatic variants and differentially expressed genes linked to predicted on- and

439 off-targets, allowing researchers to validate on-target edits and prioritize potential
440 off-targets for experimental validation ³⁷. Giandomenico Turchiano (University
441 College London, London, United Kingdom) discussed the comprehensive
442 characterization of genome stability in ex-vivo gene-edited primary cells. He
443 presented various methods for evaluating mutations, including computer-based
444 prediction of off-targets, in vitro and in-cellula DSB in DNA, in vitro base editor off-
445 targets, and chromosomal aberrations. Turchiano emphasized the importance of
446 using a combination of techniques to gain a comprehensive understanding of
447 genome stability. He introduced the MEGA approach, which utilizes multiplexed
448 droplet digital PCR (ddPCR) for mutation analysis, enabling the simultaneous
449 detection and tracking of different types of mutations induced by designer
450 nucleases. MEGA provided insights into DNA repair dynamics, quantified the
451 presence of episomal AAV DNA, and proposed RNP thresholds to optimize HDR
452 efficiency. Additionally, Turchiano discussed the association of low-frequency indels
453 with other aberrations and highlighted the findings from using the CAST-Seq
454 technology, revealing large deletions, inversions, and translocations at on-target
455 sites, including unbalanced translocations and homology-mediated events. On a
456 similar note, Toni Cathomen (Medical Center-University of Freiburg, Freiburg,
457 Germany) discussed the concept of sensitivity and specificity when it comes to off-
458 target analysis. He explained that CAST-Seq has a high sensitivity, with a limit of
459 detection of approximately 1 event in 10,000 cells. making CAST-Seq analysis more
460 sensitive than traditional translocation assays ⁴¹ Importantly, the linear range for
461 CAST-Seq analysis is 0.01% to 1%, allowing it to detect changes in the frequency of
462 events within this range. In collaboration with AstraZeneca Cathomen's team tested

463 CAST-seq in vivo edited samples using a CRISPR-Cas9 system targeting Pcsk9 with
464 a "promiscuous" gRNA⁴². They establish that out of the 99 off-target sites identified
465 by CAST-seq, 90 could be tested by NGS and 88 of them were true off-target sites
466 as further validated by using rhAmpSeq and CRISPECTOR⁴³.

467 As noted by Manuel Gonçalves (Leiden University Medical Centre, Leiden,
468 Netherlands), achieving precise GE using programmable nucleases remains
469 challenging mostly due to the prevalent repair of DSBs by non-homologous end
470 joining (NHEJ) repair pathway rather than HDR. Besides local- and chromosome-
471 wide generation of complex structural variants⁴⁴, NHEJ can yield target protein
472 imbalances and loss of cell fitness which limit the effectiveness of DSB-dependent
473 GE approaches⁴⁵. In addition, on-target DSBs are known to trigger the activation of
474 P53, which hinders the effectiveness of GE in therapeutically relevant DNA damage
475 sensitive stem cells^{46,47}. While the use of high-specificity programmable nucleases
476 can dramatically reduce the occurrence of off-target DNA cleavage, they are not
477 capable of eliminating the unintended effects caused by on-target DSB formation,
478 with a high risk of affecting the function or fitness of edited cells. In this context, the
479 Gonçalves' team is currently exploring HDR-based gene knock-in techniques that
480 rely on conventional or high-specificity CRISPR-Cas9 nickases, as single-stranded
481 DNA breaks (SSBs), or nicks, are not canonical NHEJ substrates^{48 45 49}. This research
482 builds on earlier findings from his laboratory showing that although SSBs are *per se*
483 poor HDR stimuli, simultaneous formation of SSBs at chromosomal sites and
484 matching donor DNA constructs elicits HDR-mediated gene knock-ins while
485 avoiding P53-dependent DDR activation^{50 51}. Indeed, fostering otherwise inefficient
486 SSB-dependent HDR such as by in trans paired nicking (ITPN) approaches allows for

487 seamless chromosomal editing, including at multi-copy or essential genomic tracts
488 ^{45,49}. Moreover, ITPN could also be suitable for allele-specific editing ^{52,53}, one-step
489 biallelic editing ^{48,54}, or knocking-in whole transgenes at safe harbor loci ^{45,49}.

490 **Translation of GE therapeutic platforms into the clinic**

491 As recently pointed out ⁵⁵, there are >70 GE clinical trials (with <10 sponsored
492 by EU countries; clinicaltrialsregister.eu) currently ongoing or in the recruiting
493 phase around the globe, highlighting the incredible pace at which the field has
494 advanced in the last ten years, since the development of CRISPR as a GE tool. Of
495 these trials, almost half were related to genetically modified T-cell based
496 immunotherapies, 25% to viral infections and 35% to monogenic disorders, mostly
497 affecting the hematopoietic system. One of the most exciting milestones in the field
498 is likely represented by the imminent approval by US and European regulatory
499 agencies of the first CRISPR-based medicine to treat SCD and β -thalassemia (exa-
500 cel). Clinical successes like exa-cel are bringing the potential of a GE-based
501 treatment closer to patients, however more therapeutic opportunities are needed
502 and further obstacles need to be addressed for a smooth transition from preclinical
503 research to clinical applications. These include the urgent need to establish
504 standardized protocols and procedures for GE manufacturing, delivery, and
505 monitoring in clinical trials, as well as clearing key regulatory aspects of GE research
506 and, critically, establishing a pricing framework for these innovative therapeutics.
507 The talks delivered during the "Translation into the clinic" WG session aimed at
508 discussing the state-of-the-art of GE clinical translation for different diseases

509 (depicted in **Figure 5**) and setting the stage for the development of a roadmap that
510 could guide future research and investment in this promising field.

511 A series of presentations illustrated the preclinical and clinical development of GE
512 strategies to treat genetic blood disorders, which are among the most advanced GE
513 therapeutic applications now entering the clinic. The groups of Alessia Cavazza
514 (University College London, London, United Kingdom) and Ayal Hendel (Bar-Ilan
515 University, Ramat Gan, Israel) are devising HDR-based CRISPR/Cas GE platforms to
516 treat various primary immunodeficiencies, such as Wiskott-Aldrich Syndrome,
517 Syndrome RAG2-severe combined Immunodeficiency (RAG2-SCID), and X-linked
518 SCID SCID-X1, that rely on the use of AAV6 donor vectors to introduce the
519 corrective gene into its endogenous locus in HSPCs^{34,56}. Cavazza highlighted the
520 challenges that need to be addressed when translating advanced therapies from
521 bench to bedside, and in particular the plethora of technologies that are required
522 to thoroughly assess correct manufacturing and safety of a GE product in late pre-
523 clinical studies, pinpointing the importance of establishing standardized protocols
524 and guidelines to streamline the access of these treatments to patients. The
525 development of therapeutic strategies to treat such ultrarare genetic diseases
526 entails a series of further complications, including the availability of patient samples
527 and the need to implement newborn screening programs for early diagnosis of the
528 disease to ensure therapeutic benefits from these GE strategies. In this regard,
529 Hendel's lab has established a disease modelling system for several forms of SCIDs
530 in primary human HSPCs using a multiplexed HDR platform based on CRISPR-Cas9-
531 AAV6, thus allowing to easily assess the efficacy of a therapeutic approach while

532 sparing precious patients' samples. Hemoglobinopathies such as SCD and β
533 thalassemia, are the most frequent monogenic diseases worldwide affecting the
534 production of the adult hemoglobin β -chain. The current curative treatment for this
535 condition is allogeneic HSPC transplantation, however some of its limitations have
536 pushed scientists to find alternative therapeutic options. CRISPR/Cas has emerged
537 as a powerful tool to treat hemoglobinopathies and many approaches have been
538 developed in the past few years, some of which have already reached the clinical
539 stage. Shengdar Q. Tsai (St. Jude Children's Research Hospital, Memphis, USA)
540 provided an overview of the encouraging outcomes of current clinical trials for exa-
541 cel⁵⁷ and shared his team's efforts to test an autologous genome-edited HSPC
542 therapy for SCD at St. Jude Children's Research Hospital⁵⁸. Annarita Miccio
543 (Imagine Institute, Paris, France) proposed to use BE as an alternative approach to
544 correct prevalent β -thalassemic mutations, such as IVS1-110 G>A⁵⁹. This approach
545 offers the potential to improve the efficacy and safety of autologous gene edited
546 HSPCs transplantation, which is currently limited by the safety concerns raised by
547 unwanted on-and off target events due to the cleavage of the Cas9 nuclease. Cells
548 corrected via BE showed increased levels of adult hemoglobin production, leading
549 to an improvement in the β -thalassemic phenotype. A similar approach shown by
550 Annarita Miccio is the use of BEs to generate mutations in the -200 region of the
551 fetal globin promoters that create a KLF1 activator binding site, with the aim of
552 inducing the expression of fetal hemoglobin as a universal treatment for β -
553 hemoglobinopathies. She reported that correction of patient HSPCs using BE is safe
554 and leads to reactivation of fetal hemoglobin at higher levels compared to a Cas9-
555 nuclease approach, while avoiding the generation of indels and large genomic

556 rearrangements ⁶⁰. With shared interest in hemoglobinopathies, mutation-specific
557 repair and fetal hemoglobin induction, Carsten W. Lederer (The Cyprus Institute of
558 Neurology & Genetics, Nicosia, Cyprus) also introduced the concept of tag-
559 activated microRNA (miRNA)-mediated endogene deactivation (TAMED) as a
560 potentially general therapy and research approach ⁶¹. TAMED draws on targeted
561 insertion of miRNA recognition site (MRS) tags and on endogenous miRNA
562 expression to achieve lineage-specific silencing of tagged endogenes. For the
563 abundant erythroid miR-451a and the use case of BCL11A downregulation based
564 on tagging with miR-451a cognate MRSs, Lederer's team established proof of
565 principle for TAMED, while concluding that therapeutically relevant efficiencies and
566 wider application will depend on improved donor chemistries. ^{62,63}.

567 Potential therapies employing nucleases and BE have been developed for other
568 blood disorders, such as Fanconi anemia (FA), a rare inherited bone marrow failure
569 syndrome. The main obstacle in treating this condition is the reduced number of
570 HSPCs found in these patients, however once corrected these cells can ameliorate
571 the diseases as shown in transplantation studies. Paula Rio (CIEMAT, Madrid, Spain)
572 is optimizing gene editing strategies to correct HSPCs from FA patients. The team
573 exploited BE to introduce a silent mutation in a safe harbour locus in HSPCs and
574 observed editing efficiencies up to 80%, while preserving the clonogenic and long
575 term repopulating ability of these cells ⁶⁴. They employed a parallel approach using
576 a gRNA to generate a therapeutic SNP in primary FA HSPCs achieving a 42-64%
577 frequency of correction and a proliferative advantage of corrected cells. Overall, the
578 findings demonstrate that various GE strategies can be used to correct mutations in

579 FA patients and provide promising insights for the future clinical application of gene
580 editing in FA.

581 Cancer immunotherapy is one of the most promising recent breakthroughs in
582 medicine and aims at driving the patient's own immune system to fight tumour cells.
583 Within this field, cell-based immunotherapy using T cells engineered with chimeric
584 antigen receptors (CARs) has gained momentum after the approval of several CAR-
585 T cell-based medicines for the treatment of CD19+ B cell malignancies by both the
586 FDA) and EMA ⁶⁵. GE is being utilized to optimize existing technologies, such as for
587 example to manufacture "off-the-shelf" CAR-T cell products or to increase their
588 safety, sensitivity and longevity through gene knock-out/knock-in ⁶⁶. As such, GE
589 applications in the context of cell-based immunotherapy has been the focus of many
590 talks during the meeting. Karim Benabdellah (GENyO, Granada, Spain) presented
591 the CARAML-EXO project, which aims to enhance CAR-T cell therapy for acute
592 myeloid leukemia (AML). The project aims to generate safe and effective universal
593 CAR-T lymphocytes by knocking out the T cell receptor (TCR) and PD1 genes, and
594 establishing a suitable combination of TCR/PD/PD1 knock-out CAR-T
595 subpopulations. Juan R. Rodriguez-Madoz's team (CIMA Universidad de Navarra,
596 Navarra, Spain) has developed a novel approach using CRISPR-based GE
597 technologies and virus-free gene-transfer strategies with Sleeping Beauty
598 transposons. Their aim is to generate allogeneic CAR-T cells that are depleted of
599 human leukocyte antigen class I (HLA-I) and TCR complexes. This one-step
600 generation of edited CAR-T cells has been optimized for large-scale production,
601 enabling their potential use in clinical settings. Finally, Noelia Maldonado-Pérez

602 from Francisco Martín's group (GENyO, Granada, Spain) investigated the efficacy
603 and safety of generating off-the shelf TCR knock-out (KO) ARI CAR-T cells,(ARI CAR-
604 T cells is the first academic CAR-T cell product authorized by the the Spanish
605 Agency of Medicines and Medical Devices (AEMPS) under the "hospital exemption"
606 approval pathway) Although KO leads to on-target large deletions that should be
607 monitored as a potential safety issue, the data presented by Maldonado-Perez
608 suggest that disrupting the TCR proves to be a viable strategy for producing
609 functional allogenic ARI-0001 CAR-T cells with a similar phenotype and antitumor
610 efficacy ⁶⁷.

611 Despite the potential clinical efficacy of GE combined with CAR T-cell therapy in
612 hematologic diseases, challenges such as tumour heterogeneity, immune evasion,
613 limited trafficking and persistence, adverse effects, immunosuppression, and
614 manufacturing issues restrict their potential in treating solid tumour diseases or viral
615 infections in immunosuppressed patients. Michael Schmueck-Henneresse's team
616 (Charité-Universitätsmedizin Berlin, Germany) aims to overcome some of these
617 challenges by understanding how T cells coordinate an effective immune memory
618 against virus-infected or tumour cells and how this can be specifically modified for
619 therapeutic purposes. They use CRISPR-Cas-based gene modifications to enhance
620 T-cell migration to solid tumours through transgenic expression of chimeric
621 receptors as a targeted adaptation of homing chemokine systems for CAR T cell
622 products. Both Michael Schmueck-Henneresse and Gal Cafri (Sheba Medical
623 Center, Ramat Gan, Israel) also discussed the use of effector T cells for the treatment
624 of tumours and viral infections in vulnerable patients, as well as the clinical use of

625 regulatory T cells after transplantation. On a similar note, Cristina Maccalli (Sidra
626 Medicine, Doha, Qatar) employs genomic and immunological characterization of
627 cancer stem cells to identify the mechanisms of resistance to T cell-mediated
628 immune responses in solid tumours, increasing cell-based immunotherapy efficacy
629 by selecting high affinity, antigen-specific TCRs.

630 A plethora of therapeutic GE applications for a variety of other diseases were also
631 discussed. For example, Laura Torrella (CIMA, University of Navarra, Spain) is
632 exploring the potential of GE to treat, a rare metabolic disorder that can lead to end-
633 stage renal failure and caused by mutations in the *Hao1* gene⁶⁸. Torrella developed
634 GE strategies using CRISPR/Cas9 nickases delivered by AAV to the liver of a PH1
635 mouse model to treat PH1, showing reduced oxalate accumulation and prevention
636 of renal damage. Jose Bonafont (DanausGT, Madrid, Spain) presented a GE-based
637 curative approach for recessive dystrophic epidermolysis bullosa (RDEB), a rare skin
638 genetic disorder caused by mutations in the *COL7A1* gene, which encodes the type
639 VII collagen protein (C7). Jose Bonafont used the CRISPR/Cas9 system to edit the
640 genome of patients with RDEB. Specifically, two gRNAs were used to direct the Cas9
641 enzyme to cut the DNA at two specific sites within the *COL7A1* gene, with the aim
642 to delete the exon-bearing mutation and restore the production of functional type
643 VII collagen⁶⁹. He also presented an HDR-based protocol to precisely correct the
644 mutation by using CRISPR/Cas9 in combination with AAV6-donor template delivery
645⁷⁰.

646 Lastly, two speakers addressed the use of CRISPR-based disease modelling as a
647 powerful tool for understanding disease mechanism, identifying potential

648 therapeutic targets, and testing new treatment approaches. Alejandro Garanto
649 (Radboud University Medical Center, Nijmegen, The Netherlands) is working on the
650 development of molecular therapies for inherited retinal diseases, with a special
651 focus on Stargardt disease. The team has used GE tools in different ways: a) to
652 correct or include variants to generate isogenic controls and verify pathogenicity
653 upon differentiation to human retinal models; and b) to develop a novel therapeutic
654 strategy to target pathogenic variants in *ABCA4*. Since the eye is a model organ for
655 therapeutics, the overall goal is to halt or slow down the progression of the disease
656 to improve the quality of life of the patients. Neli Kachamakova-Trojanowska
657 (Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland)
658 employed hiPSCs to study the causative molecular mechanisms of HNF1A onset
659 diabetes of the young (HNF1-MODY) which leads to increased risk for
660 cardiovascular diseases and retinopathy ⁷¹. The Kachamakova-Trojanowska's team
661 used CRISPR/Cas9 system to introduce mutations in the *HNF1A* gene in a healthy
662 donor hiPSCs line. This approach allowed them to show that endothelial cells (ECs)
663 derived from hiPSCs with mono- or biallelic mutations in the *HNF1A* gene exhibited
664 increased vascular permeability in comparison to the respective control cells ⁷²,
665 which could contribute to the endothelial dysfunction observed in patients with
666 HNF1A-MODY.

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668 **Regulatory Issues**

669 The field of somatic GE trials is rapidly advancing in Europe and the United States,
670 with a focus on cancer treatment and monogenic conditions. While the potential

671 benefits of these therapies are apparent, the understanding of associated risks is
672 still evolving. The WG on Regulatory Issues aims to facilitate the transition of these
673 therapies from the lab to clinical trials by providing recommendations for evaluating
674 their safety, quality, and addressing potential risks. During the first year of the Gene-
675 HumDI Action, two members of the Spanish Agency of Medicines and Medical
676 Devices (AEMPS) and Andalusian Network for the design and translation of
677 Advanced Therapies (RAyTA) have joined our network and more representatives
678 from national regulatory bodies of many EU countries will be recruited in the
679 following years to ensure a thorough discussion of important regulatory aspects
680 necessary for the successful translation of GE into clinical applications. Toni
681 Cathomen (Medical Center-University of Freiburg, Freiburg, Germany) focused on
682 the regulatory considerations for safety analyses of genetically engineered cell
683 products in clinical applications. Cathomen emphasized that on-target and off-
684 target aberrations are an inevitable side effect of engineering the genome.
685 Regulatory agencies therefore require comprehensive off-target analyses in non-
686 clinical studies to identify and mitigate the risk of genotoxicity. Incorporating
687 regulatory requirements into the tests used for off-target analyses helps ensure that
688 GE tools meet safety standards and increases the likelihood of regulatory approval
689 for clinical use. Establishing common standards for quality control, data processing,
690 performance parameters, and reference materials are hence crucial for accurate,
691 reliable, and reproducible off-target analyses across different developers and
692 platforms. Harmonizing these standards and parameters is essential for meeting
693 regulatory guidelines and promoting the safe and effective use of GE technologies
694 in clinical applications. María José del Pino, (AEMPS, Spain) and Gloria Carmona

695 (RA_{dy}TA) explained the different types of Advanced Therapy Medicinal Products
696 (ATMPs) in the European and Spanish regulatory context, and where currently
697 treatments based on GE are framed challenges related to ATMP regulation and
698 translation into GMP facilities. They emphasized that Spain in general and Andalusia
699 in particular a good host country and region respectively for the evaluation and
700 implementation of clinical trials based on ATMPs, with multidisciplinary teams that
701 have good experience in the evaluation and authorization of ATMP products. María
702 José del Pino discussed the importance of scientific and regulatory advice in
703 obtaining clinical trial authorization and marketing authorization for ATMPs and
704 gene therapy. The assessment and counselling process for each product is
705 conducted on a case-by-case basis, considering the unique characteristics of each
706 ATMP, therapeutic area, and disease. There are three main pathways for assessment
707 and counselling: (1) through the innovation office of AEMPS for non-profit
708 institutions, academia, universities, start-ups, biotechnology, and pharmaceutical
709 companies; (2) through EMA and its working groups, such as the Innovation Task
710 Force or the Scientific Advice Working Party, as well as programs like STARS
711 (Strengthening Regulatory Science); and (3) through the EU-Innovation Network
712 (EU-IN), led by the Head of Medicines Agencies and the EMA, which addresses
713 emerging topics requiring action by the European Medicines regulatory network.
714 In particular, the EU-IN published the Horizon Scanning Genome Editing Report in
715 2021, providing guidance on regulatory considerations for the coming years.

716 **Conclusions**

717 The GenE-HumDi network, funded COST, represents a significant milestone in
718 advancing the translation of genome editing technologies into effective treatments
719 for human diseases. By fostering collaboration among diverse stakeholders,
720 including researchers, pharmaceutical companies, regulatory agencies, and patient
721 advocacy associations, GenE-HumDi aims to reduce knowledge fragmentation by
722 harmonizing the state of the art of the GE field and facilitate the development of
723 standardized procedures and guidelines. The network's first meeting showcased
724 the recent breakthroughs and novel developments in the GE field, including
725 delivery strategies, safety considerations, clinical translation, and regulatory aspects.
726 With the recent forthcoming approvals of gene editing medicines by European and
727 American regulatory authorities, the field is rapidly progressing towards bringing
728 innovative therapies closer to patients. However, challenges remain, including the
729 need for standardized protocols related to safety, regulatory clarity, and
730 establishment of pricing frameworks. The GenE-HumDi network is strongly
731 committed to contribute significantly to overcoming these obstacles and driving the
732 future of GE as a valuable alternative for treating a broad range of human diseases.

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742 **Declaration of Interests:**

743 PR has licensed the PGK:FANCAWpre* LV medicinal product and receives funding and
744 equity from Rocket Pharmaceuticals, Inc., Patents & Royalties, Research & consulting
745 Funding. D.L. is an inventor on a patent National Institute of Chemistry filed
746 (WO/2021/032759 patent application, European patent application EP 3783104, China
747 patent application CN 114269930 with National Phase entry EP2020756868). ROB holds
748 patents related to CRISPR/Cas genome editing and has equity in Graphite Bio and is
749 consultant for UNIKUM Tx. GG holds patents related to CRISPR/Cas genome editing, is an
750 employee of CasZyme and has equity in CasZyme. S.Q.T. is a co-inventor on patents for
751 GUIDE-seq, CHANGE-seq, and other genome editing technologies and a member of the
752 scientific advisory boards of Prime Medicine and Ensoma. T.Ca. is a co-inventor on patents
753 for CAST-Seq, Abnoba-Seq, and other genome editing technologies, and a member of the
754 scientific advisory boards of Cimeo Therapeutics, Excision BioTherapeutics, and GenCC.
755 A.C. and G.T are inventors on a patent for MEGA (WO/2023/079285), G.T is also co-inventor
756 on a patent for CAST-seq.

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768

769 **Author contributions**

770 A.C. Conceptualization; data curation; Network administration, investigation; visualization;
 771 writing—original draft; writing—review and editing A.H.,, R.O.B P.R, M.G, D.L, V.A, L.P, F.Z.H,
 772 J.H, F.G.O, C.G.M, C.L, K.M., G.G, N.K, M.A.F.V.G; A.G, L.M. M.M, J.G, M.L, R.S, J.R.R.M, N.M.P,
 773 L.T, M.S.H, C.M, J.G, G.C. A.M. F.M, G.T., T.C. Y.L. T.Q.S. validation; review editing K.B.
 774 Conceptualization; funding acquisition; investigation; Network administration; resources;
 775 supervision; visualization; writing—review.

776 **Keywords**

777 European Cooperation in Science and Technology (COST); GenE-HumDi; Genome Editing; Base
 778 editors; Delivery Systems; Regulatory Guidelines

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950 **Figure Legends**

951 **Figure 1** The map illustrates the global reach of Gene Humdi COST Action, with 269
952 participants distributed across 38 countries. Each participant is actively involved in at least one
953 of the consortium's working groups

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955 **Figure 2. Gene editing technologies based on CRISPR/Cas systems.** The CRISPR/Cas
956 toolbox contains multiple versions of Cas enzymes combined with other proteins to
957 manipulate genomic DNA. For conventional genome editing, Cas9 nucleases are used to
958 create DNA double strand breaks (DSBs) that facilitate insertions or deletions (indels) of
959 base pairs at the target site introduced by DSB correction via the Non-Homologous End
960 Joining (NHEJ) pathway, leading to disruption of target DNA sequences. For precise edits,
961 Cas9 nucleases are supplemented with a DNA template for its integration into the desired
962 target locus by either homology-directed repair (HDR) or by homology-independent
963 targeted integration (HITI). These approaches are accompanied by the simultaneous
964 introduction of undesired indels, as such other approaches have fused different DNA
965 modulatory proteins to Cas9 to alter the indel spectrum or to affect the HDR:indel ratio to

966 favor HDR. The DNA break-free base and prime editors (BE and PE respectively) display
967 high product purity of the editing outcome and highly decrease the risks associated with
968 DNA DSBs, including induction of gross chromosomal aberrations. BE mediate single-base
969 substitutions, while PE can create small precise insertions, deletions, or base substitutions.
970 To induce insertion of large DNA regions, some systems utilize Cas9 fused to transposases,
971 serine integrases (PASTE) or CRISPR-associated transposases (CASTs) to insert large donor
972 DNA templates. PASTE inserts an attB site into the desired genomic location by prime
973 editing, followed by the integration of the donor DNA via the serine integrase (e.g. Bxb1)
974 acting on the flanking attP site. The CAST system uses CRISPR-associated transposases to
975 insert transposon DNA engineered to carry the desired cargo. To manipulate the
976 transcriptional status of a gene, a nuclease-deactivated version of Cas9 (dCas9) is employed
977 that maintains the ability to bind a specific DNA target. When fused to transcriptional
978 activators or repressors, target genes can be dialed up or down. By instead using fusion
979 proteins that regulate the epigenetic status of a gene, inherited epigenetic marks can lead
980 to permanent modulation of transcription.

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983 **Figure 3. Gene editing delivery systems.**

984 Schematic illustration of the varieties of the tools to deliver genome editing components,
985 classified into two categories based on the different constituents and cellular entry
986 mechanisms: viral and non-viral methods. In the first category, the most widely used viruses
987 for delivery of GE tools are Retroviruses, Adeno Associated Viruses and Adenoviruses,
988 where entry mechanisms of the gene editing components into the target cell are virus
989 specific. Viral methods can be used for both in vitro and in vivo applications. The non-viral

990 delivery methods can be further split into three subgroups: physical methods utilized for in
991 vitro gene editing (gene gun, electroporation and microinjection), and biological
992 (extracellular vesicles, EVs) or chemical (lipid nanoparticles, LNPs; Poly lactic-co-glycolic
993 acid nanoparticles, PLGA NP; dendrimers and inorganic nanoparticles) methods for in vivo
994 gene editing.

995

996 **Figure 4: Sensitive Assays and Tools for Prediction and Detection of Off-Target Sites.**

997 **A.** Schematic overview of in-cellula off-target detection methods. Upper panel, from left to
998 right: Integrase-deficient Lentivirus Capture (IDLV Capture); Genome-wide, Unbiased
999 Identification of DSBs Enabled by Sequencing (Guide-Seq); Direct In Situ Breaks Labeling,
1000 Enrichment on Streptavidin and Next-generation Sequencing (BLESS). Lower panel, from
1001 left to right: High-throughput, Genome-Wide Translocation sequencing (HTGTS);
1002 Chromosomal Aberrations Analysis by Single Targeted Linker-mediated PCR Sequencing
1003 (CAST-Seq); Discovery of In Situ Cas Off-targets and Verification by Sequencing (Discover-
1004 Seq). (DSB: double stand break; NGS: next-generation sequencing; dsODN: double-strand
1005 oligo DNA; LTR: long terminal repeat). **B.** Schematic overview of biochemical off-target
1006 detection methods, from left to right Digenome-seq, Site-Seq, Circle SEQ and Change-Seq

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1011 **Figure 5. Applications of Gene Editing in the Treatment of Inherited Rare Diseases,**

1012 **Cancer, and Infectious Diseases**

1013 Summary diagram of conditions treatable by gene editing that are currently being
1014 investigated by GenE-Humdi Cost action members. Specific gene targets are listed in italics
1015 per each disease.

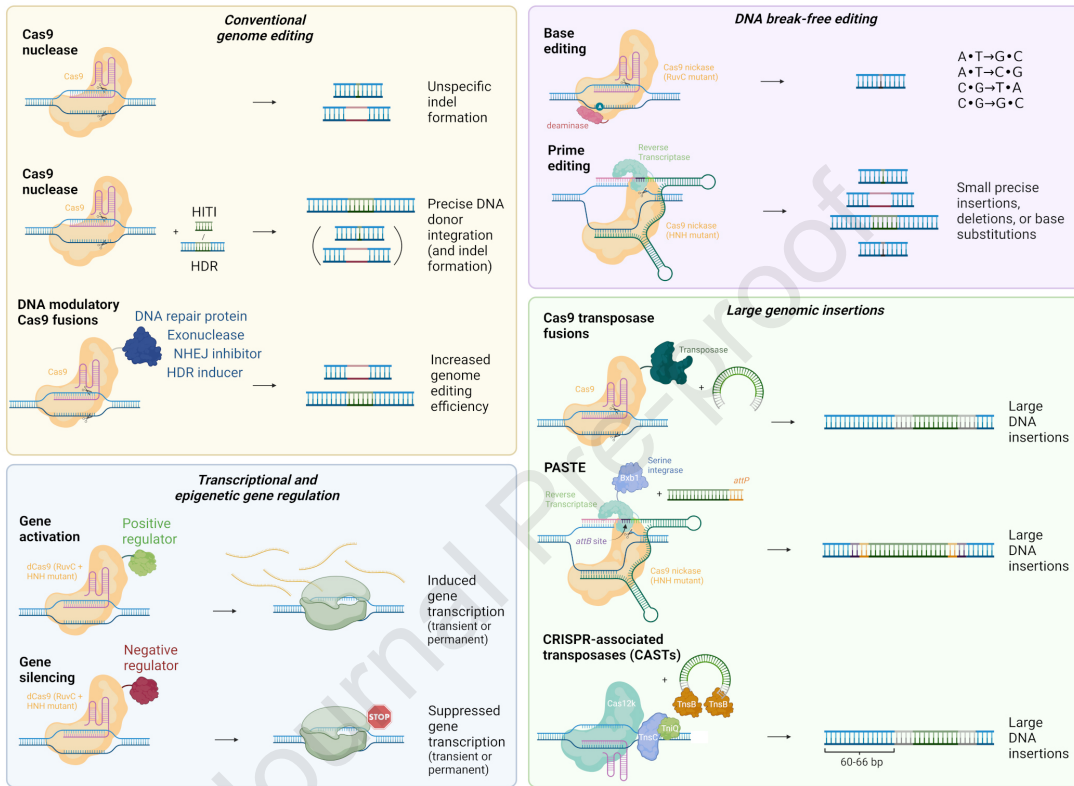
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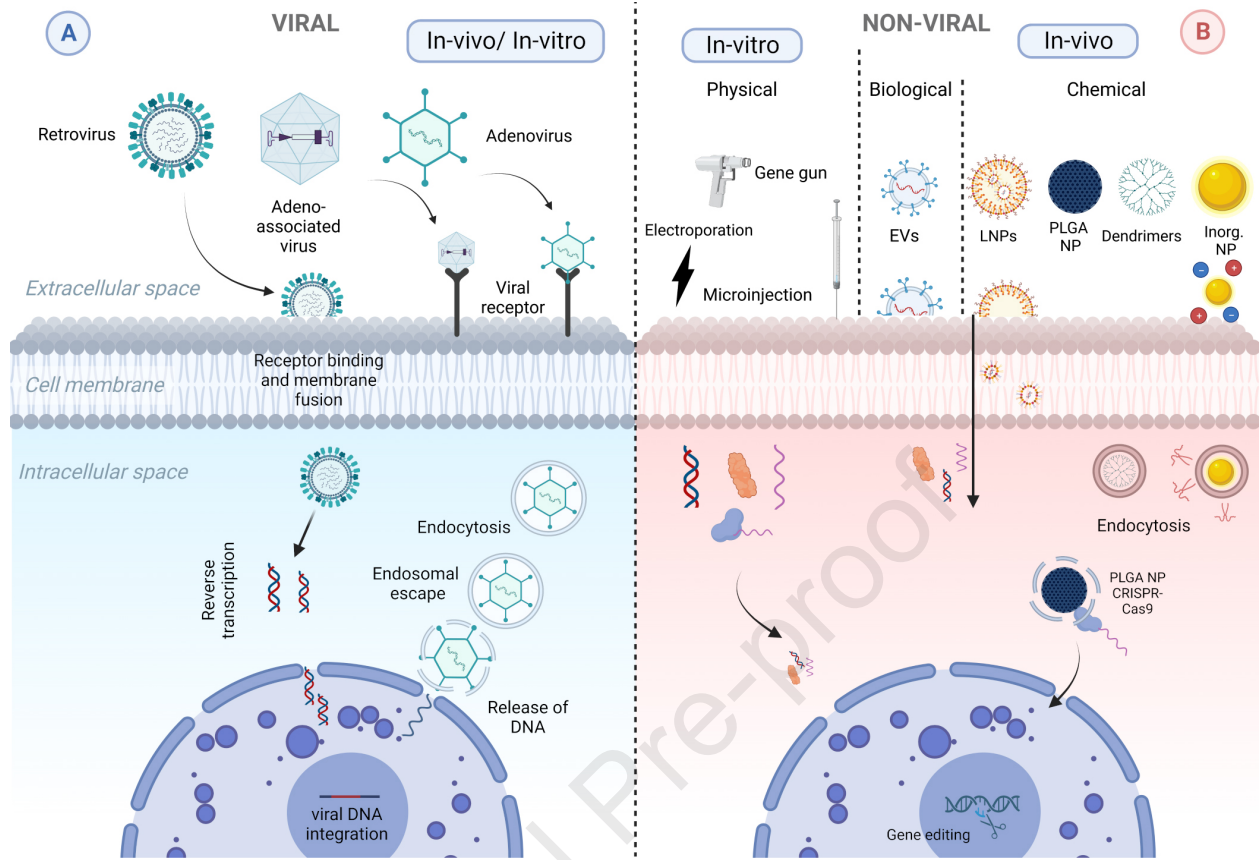
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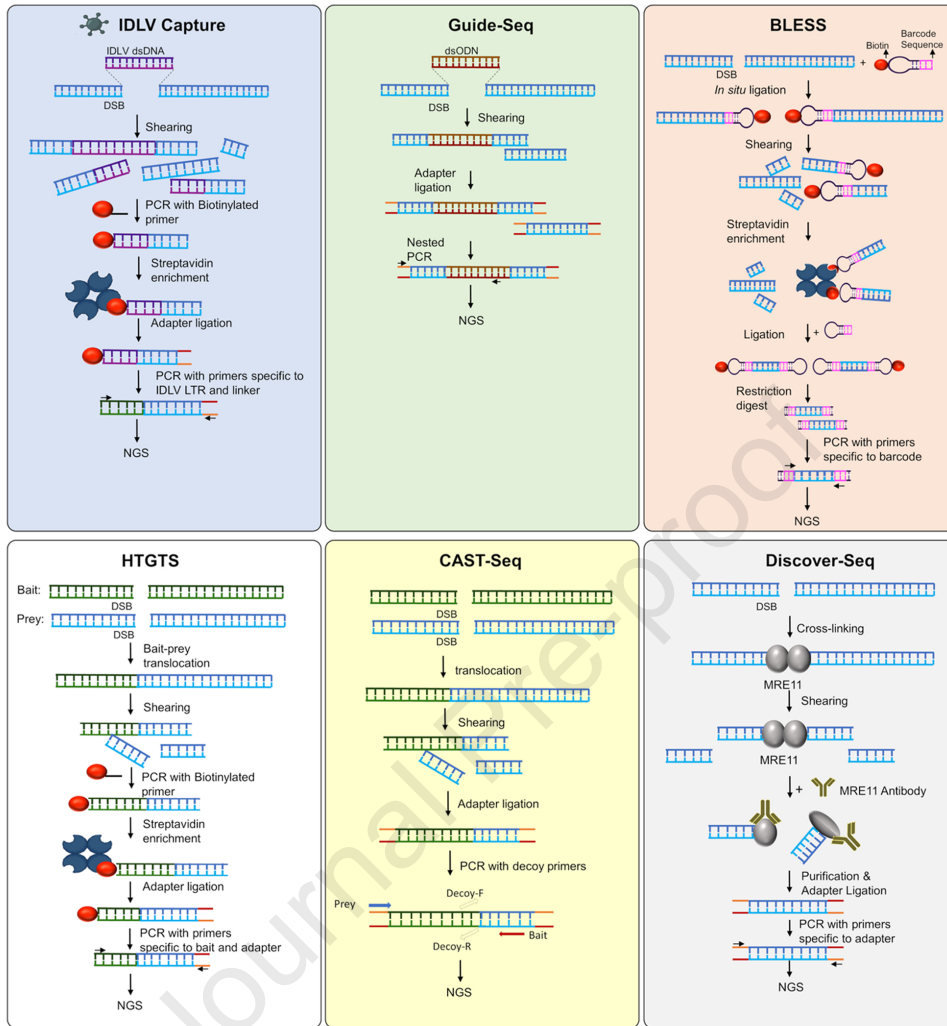
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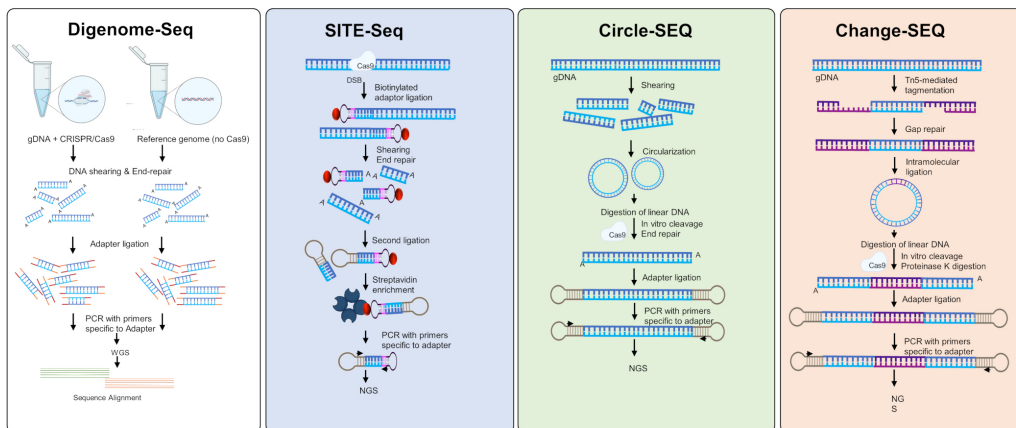
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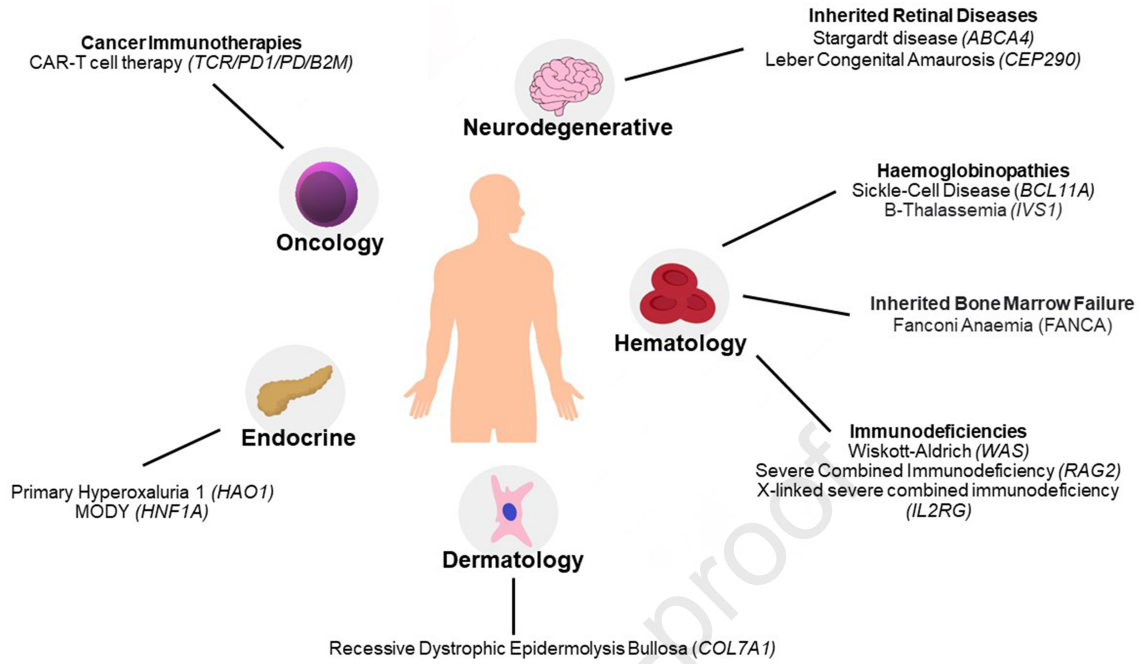


In cellula off-target detection methods



Biochemical off-target detection methods





The COST Action GenE-HumDi network unites experts, pharmaceutical industry, academia, and regulatory authorities to accelerate genome editing for treating human diseases. GenE-HumDi's mission includes refining technology, ensuring safety, and promoting accessibility.

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