REFERENCE DOCUMENT

An overview of genome editing tools and members of Working Group 2 of the GenE-Humdi COST action

AUTHORS

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INTRODUCTION

In COST action CA21113 - Genome Editing to Treat Human Diseases (GenE-Humdi), Working Group 2 (WG2) with the thematic title 'Improvement of GE technologies' was established with the aim to consolidate information pertaining to the efficacy and specificity of gene editing (GE) tools. The primary goal of WG2 is to define research priorities within the field. Hence, the activities within the working group will play a pivotal role in fostering robust collaborations among diverse GE technologies.

This internal reference document serves to address the two first deliverables of WG2 which are to provide an overview of the available endonucleases-dependent and -independent GE tools, respectively. Meanwhile the document also serves to provide an overview of the diversity or WG2 members and their research topics and expertise. The document will be shared with GenE-Humdi members on the action's website.

METHOD

The information for this reference document was made using a MentiMeter survey conducted in Dec. 2023 to Jan. 2024. All 87 members of WG2 were invited to join the survey and 46 people responded. These 46 members spanned widely in seniority with responders at the student level (undergraduate to PhD) and various independent senior scientist positions and professorships. There were also respondents from industry. A full list of the respondents, their affiliation, title/position, and country of institution/company is included in Appendix 1. The country distribution of respondents can be seen in Figure 1.

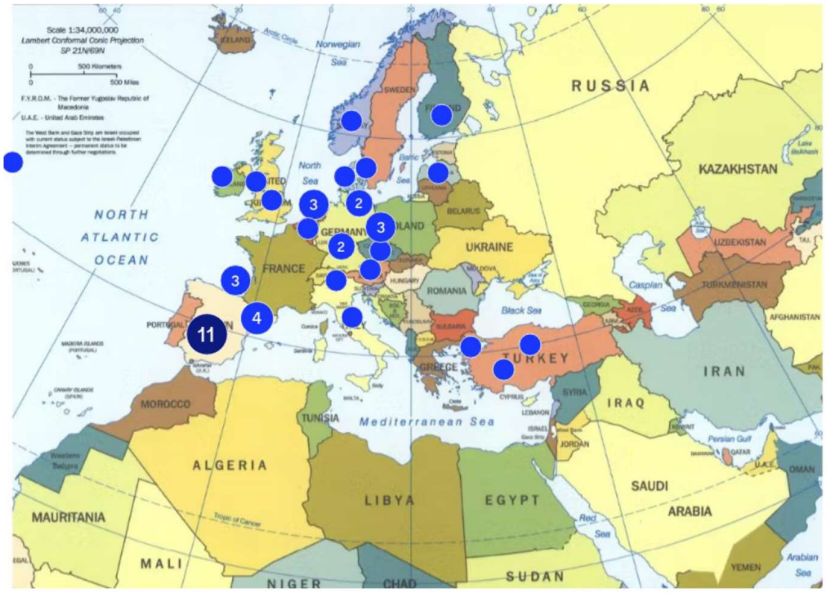

Figure 1. A representation of the countries of the affiliated institutions / companies of the respondents.

RESULTS

RESEARCH INTERESTS

The respondents were asked to list their main research interests and their responses were first binned into suitable representative words (e.g. various forms of ‘gene editing’, ‘genetic editing’, and ‘genome editing’ were grouped into ‘gene editing’). Furthermore, some focus areas such as cancer subtypes were grouped into the term ‘cancer’. The collected data shows a clear focus on gene editing using CRISPR/Cas with main research areas in cancer, stem cells, rare diseases, CAR T cells, animal models, and genetics.

Figure 2. A word cloud representing the research interests of the WP2 respondents.

RESEARCH EXPERIENCE

Next, the respondents were asked to rate the degree of experience with gene editing, and their responses, as depicted in Figure 3, show a wide distribution of experience with most respondents having mid- to high level of experience.

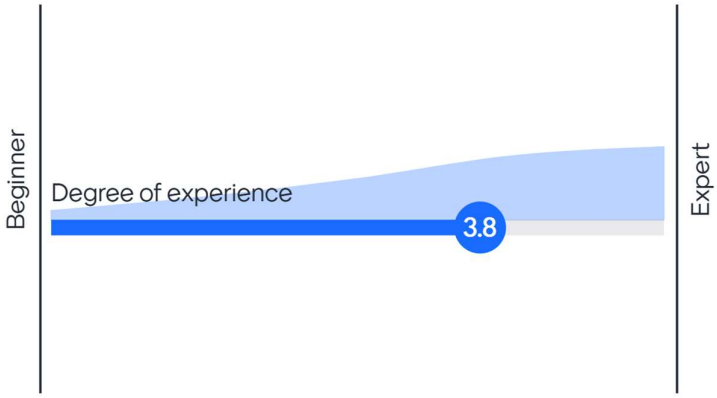

Figure 3. The degree of experience with gene editing among the WG2 members that responded to the questionnaire.

NUCLEASE-DEPENDENT VS NUCLEASE-INDEPENDENT TOOLS

As the COST action focuses on both endonuclease-dependent and -independent GE tools, the questionnaire next surveyed the use of these two tools. Results show that the majority uses nuclease-dependent GE tools, while only 10% use endonuclease-independent GE tools.

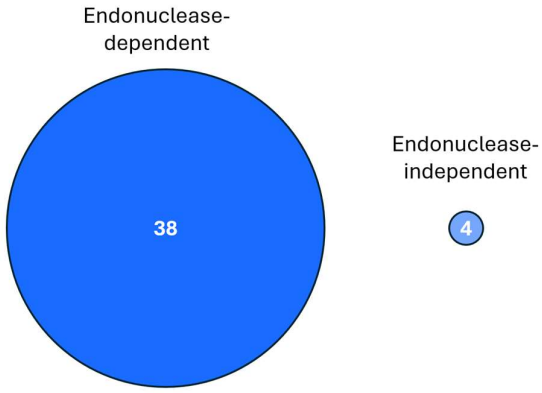

Figure 4. Distribution of researchers that work with either endonuclease-dependent or -independent genome editing tools.

GE TECHNOLOGIES USED

The respondents were next asked to list the technologies they use for either nuclease-dependent or nuclease-independent GE. Here, the respondents were ambiguous in the categorization of the GE systems for example base editors and prime editors appeared in both categories. Nevertheless, the CRISPR-Cas9 system (presumed to be the system from *Strep. pyogenes*) is the most prevalent gene editing system followed by Cas12a and Cas13. In both categories, base and prime editors are also frequently represented along with CRISPR activators and epigenetic editors.

Nuclease-dependent technologies

Nuclease-independent technologies

Figure 5. Genome editing technologies used within the categories nuclease-dependent and nuclease-independent GE tools.

EXPERIENCED EFFICIENCY AND SPECIFICITY OF GE TOOLS

WG2 members were then asked to rate the general efficiency and specificity of some mainstream GE tools, but were asked only to respond if they had experience with the tools. These results are shown in Figure 6 and show a highly rated efficiency performance of CRISPR/Cas tools for knockout and knock-in followed by base editing. However, it is important to note that CRISPR/Cas for knock-in and base editing had a bimodal distribution with some researchers stating poor editing efficiencies. This was also to some degree apparent for Prime editing. In terms of specificity, the same pattern was observed overall. It is an interesting observation that there is a direct correlation between experienced efficiency and specificity and not an apparent trade-off between these two parameters.

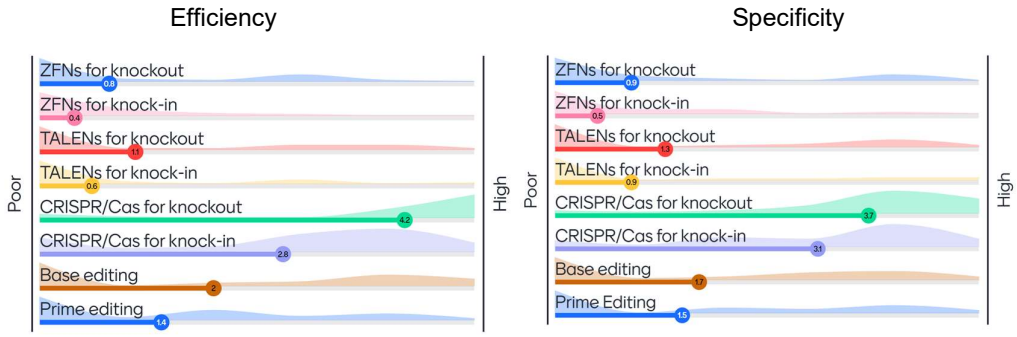

Figure 6. WG2 members rated the efficiency of mainstream genome editing tools, only if they had experience with them.

EXCITING NEW GE TOOLS

To get an insight into newly developed tools for GE and which tools might have the potential to change paradigms in the GE field,

WG2 members were asked which GE tools developed within the last two years they are particularly excited about. From Figure 7 it is evident that particularly Base editing and Prime editing are considered revolutionary new tools, although strictly speaking they have not been developed within the last two years. Other new GE tools such as PAMless Cas enzymes and recombinase technologies were also highlighted.

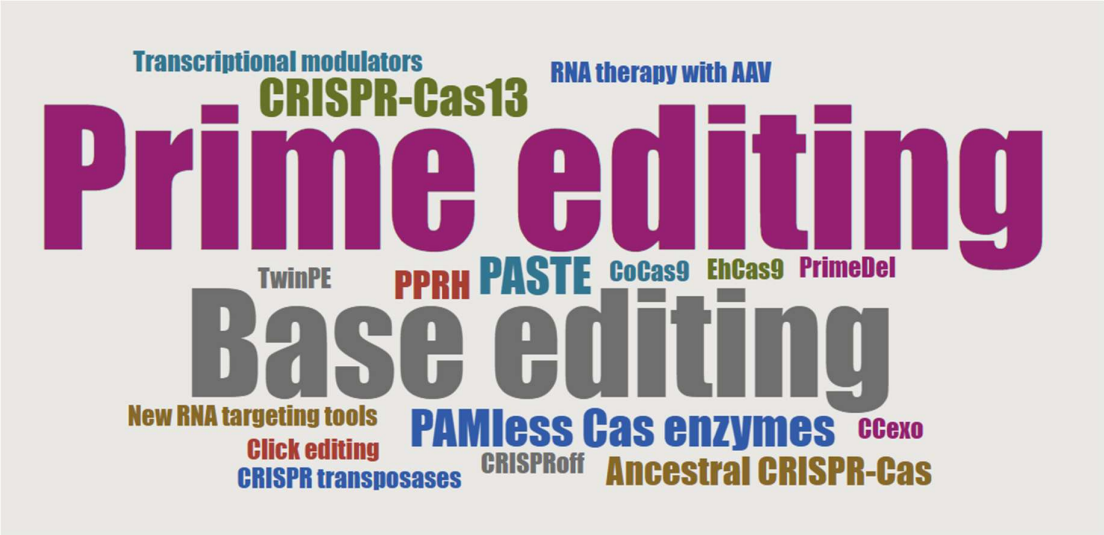

Figure 7. A word cloud representing newly developed GE tools that the respondents found most interesting

RESEARCH MODELS AND DELIVERY

The next question pertained to the research models used for GE. The respondents were asked to select up to 4 of the pre-defined options below. Cell cultures were the most frequently used research model followed by rodents and organoids (Figure 8). Only a few researchers used large animal models, invertebrates, or yeast.

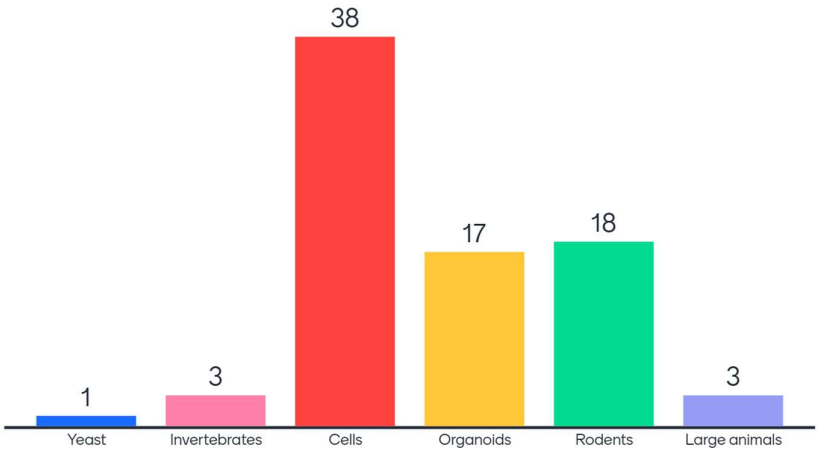

Figure 8. Research models used for GE experiments. Respondents could select up to four of the six different options.

When asked about the cell types used for GE, the responses were diverse with many different target cell types. However, hematopoietic stem cells (HSCs), T cells, induced pluripotent stem cells (iPSCs), HEK293, and fibroblasts dominated the entries (Figure 9).

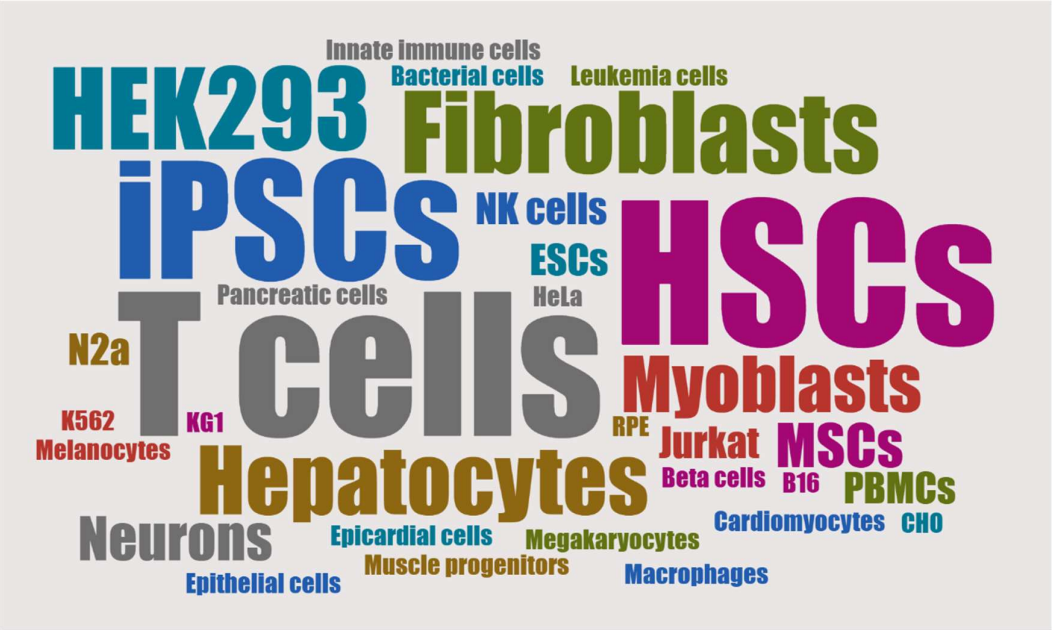

Figure 9. Cell types used for GE experiments.

For researchers performing *in vivo* GE experiments, the respondents were asked to choose up to seven target tissues from a list of options. While 16 respondents did not perform *in vivo* GE, the remaining highlighted blood and immune cells, bone marrow, liver, muscle, brain, heart, and tumors as target tissues for in vivo GE (Figure 10). Less frequent targets were kidney, pancreas, spleen, eye, and skin.

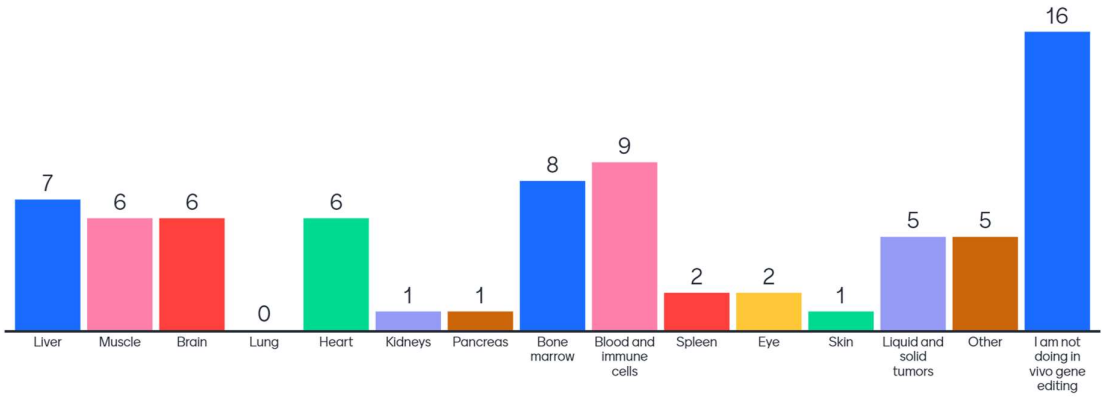

Figure 10. Target tissues for in vivo GE.

Delivery modalities are key in performing efficient GE whether it is *ex vivo, in vitro,* or *in vivo*. The responders were therefore asked to indicate the delivery systems used for their GE experiments and could choose up to four systems out of the options. Figure 11 shows that the most frequently used systems are electroporation, viral vectors, and lipid nanoparticles. Other types of non-viral modalities were also used, albeit less frequently, and these included synthetic nanoparticles, extracellular vesicles, peptide-mediated delivery, and virus-like-particles.

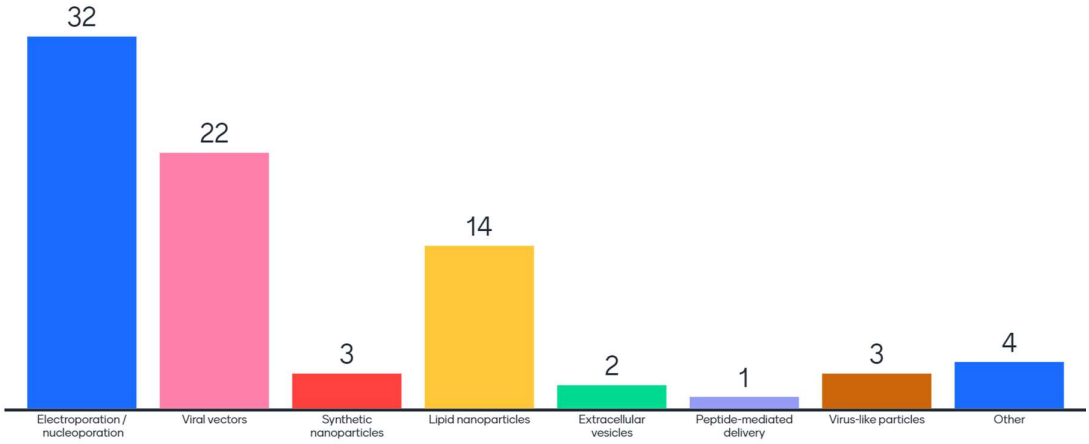

Figure 11. Delivery systems used for GE.

POTENTIAL CONTRIBUTIONS TO THE COST ACTION

The authors were asked to provide links to their AddGene plasmid repository websites if they had such. AddGene distributes deposited plasmids for a small handling fee and the plasmids are shared under a standard material transfer agreement (MTA). These website links can be found in Appendix 2.

The respondents were then queried about additional resources they could potentially contribute within the COST network, revealing a strong willingness to share with other researchers. Predominantly, these resources included protocols, plasmids, and cell lines.

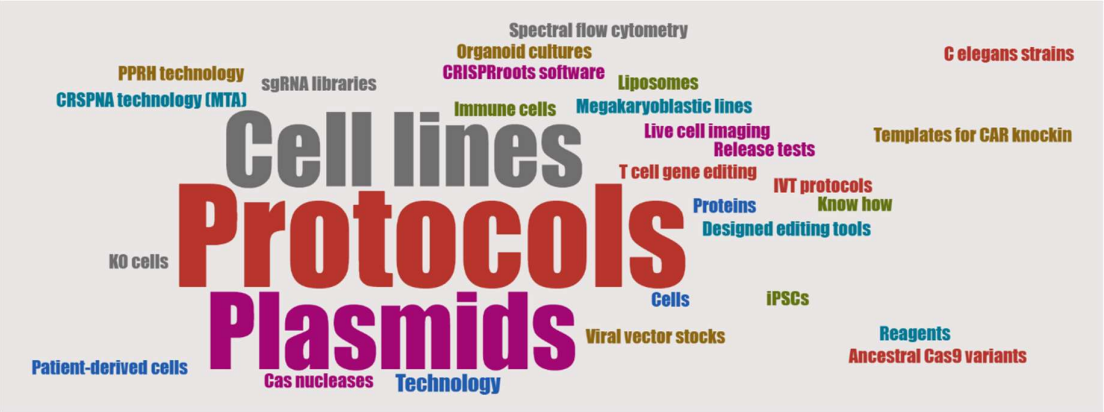

Figure 12. A wordcloud representing the resources that respondents are willing to share with other COST action members

The respondents were asked if they would be willing to host a researcher for a short research stay in their team (short-term scientific mission; STSM). These STSMs are funded by the COST networks and calls are made regularly. Most respondents answered Yes or Maybe, indicating a high willingness to host researchers in their laboratories (Figure 13). The responses included a mark on a map to indicate the location of potential host laboratories. This indicates a wide distribution throughout European countries where STSMs are possible.

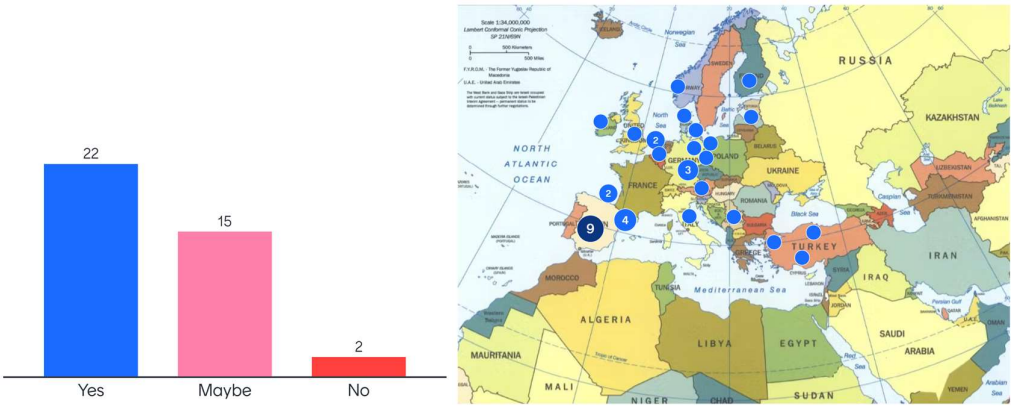

Figure 13. Willingness of the respondents to host a researcher for a short research stay; short-term scientific mission (STSM).

DISCUSSION

The rise of genome editing (GE) technologies has transformed biomedical research, offering new ways to tackle human diseases. Working Group 2 (WG2) within the COST action CA21113 - Genome Editing to Treat Human Diseases (GenE-Humdi) aims to advance GE tools and set research priorities.

WG2 includes individuals from different academic backgrounds and industries, with different degrees of expertise. This diversity fosters collaboration and brings a wide range of proficiency and viewpoints. The global representation within WG2 highlights the international collaboration in GenE-Humdi. Research interests in WG2 mainly focus on CRISPR/Cas-based gene editing, covering areas like cancer, rare diseases, and immunotherapy. WG2 members have a significant level of experience, providing a solid foundation for collaboration. Nuclease-dependent systems, like CRISPR/Cas9, are preferred for genetic manipulation due to their versatility and reliability. However, variability in efficiency underscores the ongoing need for optimization and standardization of protocols. WG2 members recognize the effectiveness of CRISPR/Cas systems, especially for modifying genes, although there are some differences among the responses. Excitement surrounds emerging GE technologies like base editing and prime editing, offering precise nucleotide-level modifications with transformative potential for therapeutic interventions. WG2 members use a variety of models, targeted tissues, and delivery methods for their GE research, showing the broad applicability of GE. They are also willing to share resources and host researchers for short-term projects, promoting collaboration within GenE-Humdi.

WG2's diverse expertise and collaborative spirit drive advancements in CRISPR/Cas-based genome editing, while a focus on refining efficiency and specificity underscores the group's commitment to advancing therapeutic possibilities within the GenE-Humdi action.

This reference document compiles information that points to base editing and prime editing as the next-generation tools to be prioritized for Genome Editing Advanced Therapy Medicinal Products (GE-ATMPs). These cutting-edge techniques offer precise and targeted modifications to the genetic code, allowing for potential breakthroughs in treating human diseases with unmet clinical needs. By harnessing the power of base editing and prime editing, researchers and clinicians can develop innovative therapies that hold great promise for improving human health and well-being.

In summary, WG2's valuable insights into GE technologies and research priorities within GenE-Humdi align closely with the initiative's primary objective of expediting the application of genome editing for therapeutic purposes in treating human diseases. Through collaborative efforts and shared expertise, WG2 aims to drive progress in GE therapies, furthering the goal of addressing human health challenges through genome editing.

APPENDICES

APPENDIX 1. OVERVIEW OF RESPONDERS

Name	Affiliation	Title/position	Country of institution / company
Laura Torella	CIMA, University of Navarra	PhD candidate	Spain
Francisco Martin	Universidad de Granada - GENYO	Principal Investigator Gene & Cell Therapy Group	Spain
Rasmus O. Bak	Department of Biomedicine, Aarhus University	Associate Professor	Denmark
Urszula Oko	Aberystwyth University	Undergraduate Microbiologist	United Kingdom
Cristina Eguizabal	Basque Center for Blood Transfusion and Human Tissues-Biobizkaia Health Research Institute	Head of Advanced Therapies-Group Leader of Cell Therapy, Stem Cells and Tissues	Spain-Galdakao (Bizkaia)
Diego Balboa	University of Helsinki	PI	Finland
Julian Grünewald	Technical University of Munich	Assistant Professor of Gene Editing	Germany
Dimitrios Laurin WAGNER	Charité - Universitätsmedizin Berlin, Germany	Group Leader	Germany
Dimitra Micha	Human Genetics department, Amsterdam UMC, The Netherlands	Assistant professor/ principal investigator	Netherlands
Gonzalo Martinez Navajas	GENyO	Postdoctoral researcher	Spain
Ayla wyninckx	2nd laster student biomedical sciences	Student	Belgium
Giedrius Gasiunas	CasZyme	CSO	Lithuania
Ignacio Perez de Castro	Instituto de Sald Carlos III	Group Leader	Spain
Omer Aydin	Erciyes University	Assoc. Prof / Research Group Leader	TURKEY
Manuel A.F.V. Goncalves	Leiden University Medical Centre	Associate professor	The Netherlands
Francisco Javier Molina Estevez	GENyO	Postdoctoral Research Fellow	Spain
Sunil Martin	Department of Biotechnology, Government of India	Scientist E-II /Adjunct Associate Professor	India
Lluis Montoliu	National Centre for Biotechnology (CNB-CSIC) and CIBERER-ISCIII, Madrid, Spain	CSIC Research Scientist	Spain

Serif Senturk	Dokuz Eylul University & Izmir Biomedicine and Genome Center	Professor & Research Group Leader	Türkiye
Miguel Moreno-Mateos	Andalusian Center for Developmental Biology (CABD), Pablo de Olavide University/CSIC/Junta de Andalucía.	Group Leader	Spain
Guillaume Pavlovic	Genetic Engineering and Model Validation Department, Institut Clinique de la Souris-PHENOMIN- IGBMC, Illkirch, France	Head of Unit, Genetic Engineering and Model Validation Department	France
karim Benabdellah	Fundación Publica Progreso y salud	PI	Spain
Lorea Blazquez	Biogipuzkoa Health Research Institute	Principal Investigator	Spain
Roman Jerala	National Institute of Chemistry, Ljubljana, Slovenia	department head	Slovenia
Rosario Fernandez Godino	Foundation MEDINA	Head of Screening and Target Validation	Spain
Alessandro Michienzi	University of Rome Tor Vergata	Associate Professor	Italy
Ernst Stefan Seemann	University of Copenhagen Faculty of Health and Medical Sciences Department of Veterinary and Animal Sciences	Associate Professor	Denmark
davide seruggia	St. Anna CCRI, Vienna	PI	Austria
Lucie Peterkova	Charles University Prague, Czech Republic	Postdoc	Czech Republic
Julián Cerón Madrigal	Bellvitge Biomedical Research Institute (IDIBELL)	Principal Investigator	Spain
Fisnik Asllani	University of Prishtina "Hasan Prishtina"	University assistant	Republic of Kosovo
Berta de la Cerda	CABIMER-FPS	Senior researcher	Spain
Iris Ramos Hernández	Genomic Medicine Department. GENYO, Centre for Genomics and Oncological Research, Pfizer-University of Granada-Andalusian Regional Government.	PhD student	Spain
Claudio Mussolino	Medical Center - University of Freiburg Institute for Transfusion Medicine and Gene Therapy	Group Leader	Germany

Michael Schmueck-Henneresse	Berlin Institute of Health (BIH) BIH Center for Regenerative Therapies (BCRT) Charité - Universitätsmedizin Berlin	Group Leader and Head Research Field	Germany
Karolina Skvarova	Second Faculty of Medicine, Charles University, Prague, Czech Republic	Principal Investigator	Czech Republic
Araceli Aguilar-González	Genyo/University of Granada	Young Posdoctoral investigator	Spain
Ciaran Lee	University College Cork	Lecturer	Ireland
Carlos J. Ciudad	University of Barcelona	Full Professor- Emeritus	Spain
Alberto Malerba	Royal Holloway University of London	Lecturer in gene therapy	United Kingdom
Veronica Noe Mata	School of Pharmacy and Food Sciences University of Barcelona	Full professor of Biochemistry and Molecular Biology	Spain
Kulbhushan Sharma	Project Investigator and Leader, Stem cell facility, Akershus University Hospital, Lørenskog, Norway	PI and leader	Norway
Alfredo Silva	Shortly will be affiliated to San Raffaele University	Student (shortly fellow researcher)	Italy
Lucie Peterkova	CLIP, Second Medical Faculty, Charles University, Czech Republic	Postdoc	Czech Republic
Dilara AKCORA YILDIZ	Burdur Mehmet Akif Ersoy University, Science & Art Faculty, Biology Department	Assist. Prof.	Türkiye
Alba Olaso Llorca	Bellvitge Biomedical Research Institut (Barcelona, Spain)	Research Assistant	Spain

APPENDIX 2. ADDGENE LINKS

https://www.addgene.org/Rasmus_Bak/
https://www.addgene.org/Timo_Otonkoski/
https://www.addgene.org/Dimitrios_Wagner/
https://www.addgene.org/Manuel_Goncalves/
https://www.addgene.org/Miguel_Angel_Moreno-Mateos/
https://www.addgene.org/Roman_Jerala/
https://www.addgene.org/Julian_Ceron/