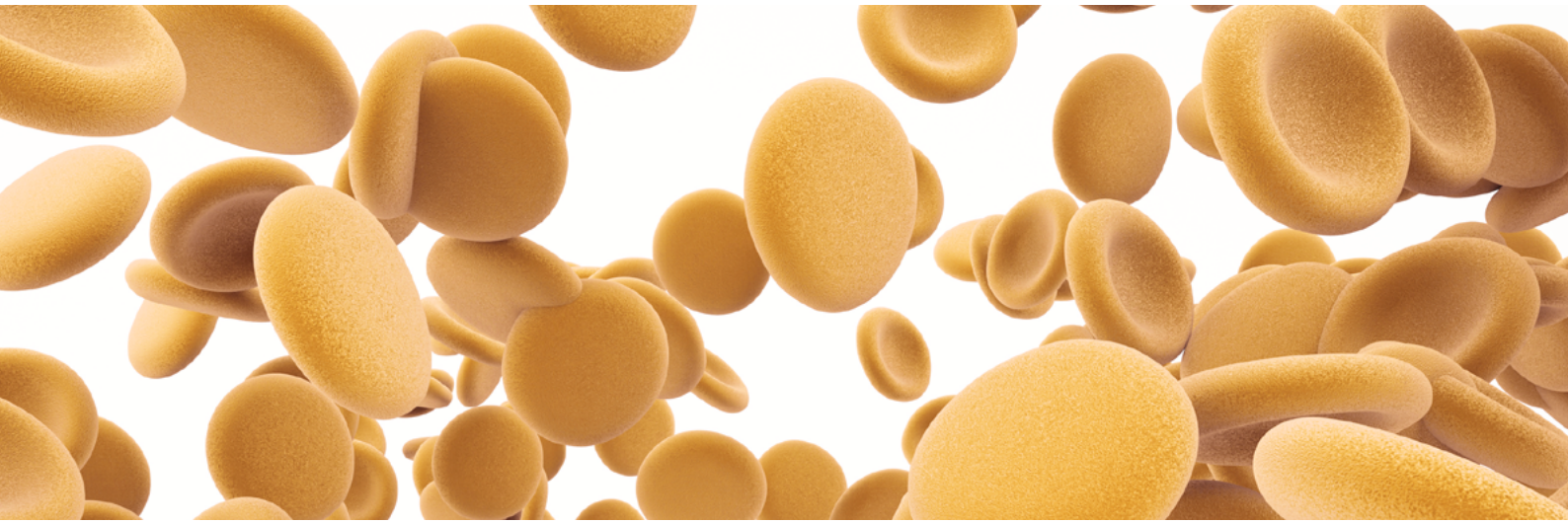
White paper on CAR-T cell therapy development:

regulatory issues and challenges to harmonize translation from bench to bedside



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1 | CAR-T cells and EURE-CART project

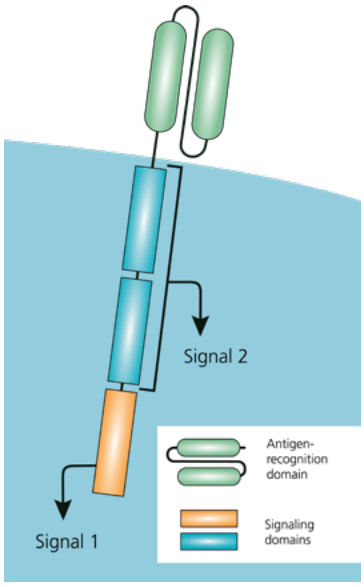
From the early 2000's cancer has become one of the leading causes of mortality in most European countries, both in men and in women. Moreover, excluding injuries, cancer is by far the first mortality cause in children.

Use of immunotherapy in clinical management of many patients with a broad variety of solid and hematological malignancies has represented a significant breakthrough that has modified the prognosis of numerous cancers that until recently would have been rapidly lethal.

Chimeric Antigen Receptor - T cells (CAR-T) are T-lymphocytes genetically engineered to produce an artificial (chimeric) T-cell receptor targeted at a specific surface tumor protein, thus enabling the T cells to specifically target and kill the tumor expressing that protein. The chimeric receptor combines both antigen-binding and T-cell activating functions into a single receptor. In this strategy, T cells can more effectively target cancer cells to destroy them.

Many potential CAR-T targets exist on the surface of tumor cells but some of these targets can be lost or mutated by the tumor under the immune-pressure and/or can be expressed also by non-malignant cells leading for example to the risk of severe on target off tumour toxicity. Thus, although CAR-T should be considered a highly adaptable platform and several types of CAR-T can be potentially produced, their clinical development is not trivial in particular due to the potential risks related to the activation of powerful T cell responses against a defined target.

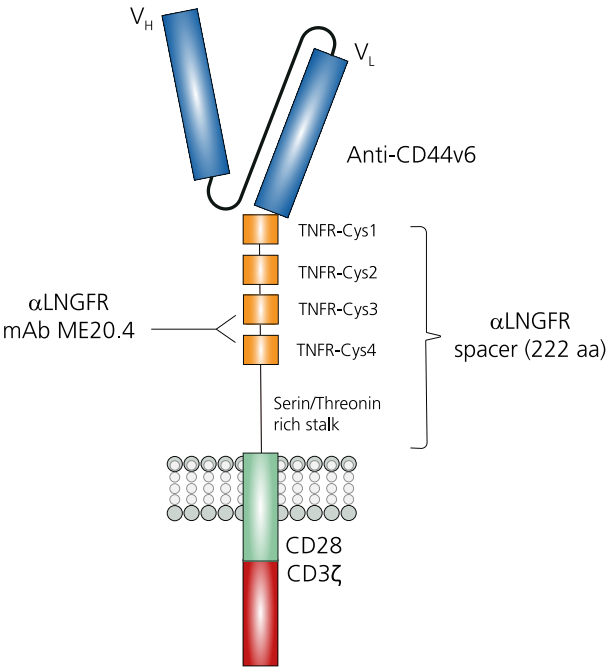
Fig. 1 shows the structure of a Chimeric Antigen Receptor (CAR).



Very encouraging results have recently been obtained with engineered CAR-T cells [1-5], leading to first market approvals in 2018 in the European Union (EU) for two CAR-T cell medicinal products (Yescarta® and Kymriah®) for B cell lymphoma and B cell acute leukemia therapy. The CAR-T products were also approved for marketing in other world market areas such as USA and Asia. Both these products are directed against the B cell malignancy surface antigen CD19. Many other clinical trials are presently carried out with these two products for other cancer indications and other CAR-T cells targeting different tumor antigens (684 worldwide, 78 in EU; [6]). Based on the clinical data, the two currently available CAR-T products are expected to rapidly extend for market authorizations to other indications such as multiple myeloma (anti BCMA CAR-T – two products), mantle cell lymphoma (KTE-X-19) and possibly indolent lymphomas, in the next future.

EURE-CART (**EUR**opean **E**ndeavour for **C**himeric **A**ntigen **R**eceptor **T**herapies) is a research project supported by EU by means of a Horizon 2020 grant. In the EURE-CART project, the v6 variant of adhesion molecule CD44 was chosen as the tumor target antigen. CD44v6 was first described as a metastatic factor engaging in cancer progression. It is expressed in acute myeloid leukaemia (AML) and multiple myeloma (MM) and is associated with a poor prognosis [7-8].

Fig.2 shows the structure of CD44v6.CAR.



EURE-CART project aims at developing CD44v6.CAR T cells as a medicinal product and performing a European multicenter phase I/II clinical trial with the CD44v6.CAR-T cells in the patients affected by relapse/refractory AML and MM. AML and MM were chosen because the existing therapies often increase patient survival but fail to achieve a definitive cure, thus an efficacious medicinal product presents an urgent clinical need. The European Member States (EU MS) involved in the clinical development are Italy (IT), Spain (ES), Germany (DE) and Czech Republic (CZ).

The phase I/II clinical trial will allow to obtain a proof-of-concept of immunetherapy with CD44v6.CAR T cells and provide the basis for further developing CD44v6.CAR T cell therapy in AML and MM.
In addition, it will provide data that will be useful to plan CD44v6.CAR T cell therapy for other tumors that express CD44v6, including breast, colorectal, pancreas, head and neck cancer that affect the life of hundreds of thousands of EU citizens.

The project aims at a clinical trial, an activity not only with a very high scientific content but also highly regulated. A broader aim of the project is to contribute to the efficient development of new therapies based on cells and genes. Therefore, the scientific and management structure of the project include a regulatory package (WP3) aiming at giving regulatory advice during the project development and in the end contributing to streamline the regulatory pathways for CAR T cell products in EU. WP3 also includes a Regulatory Advisory Committee (RAC) independent from the scientific work of the other units of the project. European experts in gene therapy regulatory field together with scientific experts are represented in the RAC.

The most critical regulatory issues, as highlighted to be addressed during the development of CD44v6. CAR T cell therapy, pertain to the following aspects: clinical trial authorization process and Genetically Modified Organisms and Micro-organisms (GMO/GMMO) issues. This white paper represents the output of WP3 work during the project and describes the regulatory challenges faced to bring the CD44v6. CAR T cell therapy from laboratory to patients, highlighting areas where European harmonization is still to be achieved and suggesting possible ways to reach it.

2 | Current EU regulatory frame for the authorization process of a clinical trial with CD44v6.CAR-T cells

According to European legislation, CAR-T cells are classified as a gene therapy medicinal product (GTMP). As an example, classification recommendations made by the Committee for Advanced Therapies (CAT) of the European Medicines Agency (EMA) can be consulted in the EMA website [9].

The CD44v6.CAR-T cells is therefore an investigational gene therapy medicinal product (iGTMP) to be developed and used in a first-in-man clinical trial. Clinical trial authorization process in EU is still regulated according to the Dir.2001/20 [10], even though EU Regulation 536 [11] was issued in 2014 requiring European centralized assessment of a clinical trial application. Until the EMA portal is made active, the EU Reg.536 cannot be implemented. Therefore, assessment and approval for a given clinical trial are still carried out at national level, which means that a single approval procedure is separately required from each EU MS where the clinical trial takes place. In each EU MS, at least two different Competent Authorities (CA) are involved: the Medicines Agency and the local Ethical Committee (EC). The application procedures are specific to each of the CAs. In this project, four EU MS, that is IT, ES, DE, CZ, are included in the clinical trial, so there would be 4+4 procedures for the 4+4 CA in order to get 4+4 final opinions from four EU MS.

In addition to the clinical trial approval by the CA, the sponsor needs to negotiate with individual hospitals on the costs for enrolling the patients. The EU Directive 2001/20 [10] lays down a common procedure (application format, deadlines, need for a written opinion) for the clinical trial approval process. It is the responsibility of each CA to identify applicable scientific requirements and to issue relevant guidelines, if they deem it necessary. Many CA refer to EMA guidelines, even though those are generally addressing the requirements at the market authorization level and not at the clinical trial level, particularly not for first in man trials. Noteworthy exception is the guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMEA/CHMP/GTWP/125459/2006) [12] EMA CAT is in the process of issuing another guideline that addresses the requirements for ATMP in clinical trials [13].

In conclusion, requirements for GTMP development and translation into first-in-man clinical studies are not harmonized at European level, leaving a certain degree of uncertainty in the approval process outcome in the case of a clinical trial to take place in several European countries, such as the one described in this project.

With the aim at overcoming this dis-harmonization until the EU Reg.536 [11] is implemented, a Voluntary Harmonization Procedure (VHP) has been made available at European level to obtain a coordinated assessment of an application for a European multinational clinical trial [14;15]. On a voluntary basis both for the sponsor and for the concerned EU MS, a single dossier is presented in one single procedure to the EU MS involved in the European multinational clinical trial. The involved EU MS

elaborate a common opinion on the acceptability of the clinical trial. By entering in this procedure, the clinical trial sponsor receives a common opinion from the Medicine Agencies of the EU MS involved in the European multinational clinical trial, after which, if positive, formal approval by the individual EU MS is still required but with a quicker timeline as compared to national procedure. The VHP does not cover the EC assessment of the clinical protocol nor the GMO/GMMO assessment, that still need to be carried out at national level.

3 | GMO/GMMO issue

The CD44v6.CAR T cells are genetically modified cells; therefore, the iGTMP should fulfill also the European requirements laid down in the EU legislation for GMMO (EU directive 2009/41 on contained use) [16] and for GMO (EU directive 2001/18 on deliberate release [17]. These legislations require GMMO/GMO procedures and approvals at national level, but are not applicable at the market authorization procedure level. The EU Regulation 726/2004 [18] covers the market authorization steps for a medicinal product containing GMMO or GMO, stipulating that GMO/GMMO aspects are handled by the pharmaceutical CA within the environmental risk assessment required for the market authorization process. Therefore, the separation between the directives on GMO/GMMO and the EU Regulation 726/2004 remains for the clinical trial development stages. The directives on GMO/GMMO are not referred to in Directive 2001/20 nor in the Regulation on clinical trials and, symmetrically, the legislation on clinical trials with iGTMP is not referenced in the GMO/GMMO directives. The result is that during iGTMP development the respective application and approval schemes for GMO/GMMO and for a clinical trial are completely independent in each EU MS. In most EU MS the two procedures are in parallel, while in 5 EU MS (Poland, Romania, Bulgaria, Slovakia, Slovenia) the GMO/GMMO authorization should be obtained before clinical trial application. In 5 EU MS (Germany, Sweden, Lithuania, Estonia, Greece), a single application for both GMMO/GMO and clinical trial authorizations is required. Added levels of variability among EU MS derive from which directive is applied by different CA. Some EU MS apply the directive on GMO, others apply the directive on GMMO and finally other EU MS choose which directive to apply on a case-by-case basis. CA involved may be the Ministry of Environment or the Ministry of Agriculture or the Ministry of Health. The assessment time is also very variable, from less than 30 days to almost 90 days (Repository of national requirements in [19]). The net result is that there are 27 different procedures and CA to face for the use of iGTMP. It may happen that in a EU MS the GMMO/GMO contained in the iGTMP is approved under the environmental point of view, but its clinical use is considered to be too risky for the patients and thus not approved; while on the contrary, in another EU MS the same iGTMP for the same clinical trial may be approved for use in humans, whereas its environmental risk is considered to be too high and its handling or release is not authorized. In order to address this complex situation raised in the past years and many protests from the developers, the EC convened in 2017 an ad hoc Working Group (WG) on the interplay between the GMO legislation and the legislation on medicinal products. The WG's scope was on clinical trials with GMOs, including the clinical trials with genetically modified human cells by means of retro/lentiviral vectors. The WG developed a simplified procedure including a common application form and a Good Practice document on the assessment of GMO-related aspects in the context of clinical trials with viral vectors and with human cells genetically modified by means of retro/lentiviral vectors. The document was endorsed by many EU MS (Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Portugal, Romania, Spain, Sweden and Norway).

In the EURE-CART project, four MS (IT, ES, DE, CZ) are included. Even with a GMO common application form and a simplified procedure, still there would be 4 procedures, 4 CA and 4 outcomes. This is an added level of challenge faced during the development of the iGTMP CD44v6.CAR T cells.

4 | Challenges faced during EURE-CART: VHP, GMO, lack of harmonization between EU Competent Authorities

As described above, clinical trial authorization process in EU is still under the Dir.2001/20 [10]. Therefore, assessment and approval for a given clinical trial are still carried out at national level. EU Directive 2001/20 lays down a common procedure for the clinical trial approval process, however it does not describe common requirements for quality, safety and efficacy of the investigational medicinal product (IMP). Assessors from National CA (NCA) do not work together in the clinical trial evaluation process even for a multinational trial. Assessor training at EU level in each section of the IMP characteristics (quality, safety and efficacy) has been carried out in past years only with a view at market authorization level (EMA/CAT/654949/2012 Activities proposed by CAT-IP Focus group on non-clinical development of ATMPs; [20], based on the fact that EMA remit is not on clinical trials.

The consequence is the possibility of dis-harmonization between NCA in assessing a clinical trial application. To overcome this risk of dis-harmonization, the RAC advised applicants to use the VHP. VHP is available at the Clinical Trial Facilitation Group established among EU MS on a voluntary basis. This procedure is designed to offer the applicant a single assessment of an EU multinational clinical trial, made by the participant EU MS who work together in the assessment process. In the VHP, only one opinion is given to the applicant about the acceptability of the EU multinational clinical trial application. This opinion is not the formal approval, the applicant still needs to approach individual NCA for the clinical trial approval; however, once a positive VHP opinion is granted, the formal approval is easily obtained.

EURE-CART participants thus approached the VHP for the clinical trial to be approved, prepared the required documents in English language: Investigational Medicinal Product Dossier (IMPD) (quality and non-clinical data) and Clinical Study Protocol. It was a difficult process, as it took several weeks just to obtain from the VHP rapporteur the information that the EURE-CART clinical trial was not eligible because the timelines of the VHP cannot be fulfilled in the case of a GMO-based ATMP such as the IGTMP involved in the EURE-CART clinical trial. The differences in EU MS for the GMO process would negatively impact on the VHP. This was not expected, as in the VHP note for applicants there is no mention of this case of non-eligibility. In other words, the application to VHP was rejected in Oct 2018 because of the GMO legislation.

NCA application was then the only choice for obtaining the clinical trial approval. An application for a clinical trial entails submission to NCA of the following documents: IMPD (quality and non-clinical data), Clinical Study Protocol, Investigation Brochure, Informed Consent Form, Study Manual, Administrative Contracts to the Clinical Centres. Each should be in the national language, some EU MS accept documents in English language (e.g. Italy). It is evident that producing all those documents is a huge workload that was not expected in the project timing and it caused a significant delay.

In parallel, the GMO procedures were started in all the concerned EU MS for all the hospitals involved. Fortunately, many of the participants had already obtained the required GMO authorizations. This fact was not taken into consideration in the VHP process. EURE-CART clinical trial application was submitted in Italy, Czech Republic, Spain and Germany. Each NCA received the very same documents as the others. Approval was granted only by Italian and Czech NCA. When the sponsor's answers to the questions raised by assessors in the first assessment were assessed (a process that took several months), Spanish and German NCA still refused the approval on the basis of preclinical safety issues that had been considered resolved by the Italian and Czech NCA. Refusal from Spanish and German NCA is also on the basis of different issues that are considered still open.

As stated above, the legislation and the procedures on clinical trial application in EU can raise the possibility of dis-harmonization between NCA in assessing a clinical trial application. This indeed has been the experience for the EURE-CART project clinical trial.

5 | EU funded projects involving a clinical trial: project external aspects impacting on timelines, deliverables, and who can affect them

One of the EURE-CART project deliverables is the multinational clinical trial with the CART IMP to be carried out in 4 EU MS: Italy, Spain, Czech Republic and Germany. As described above, this has not been completely achieved as two concerned NCA refused the approval to the clinical trial.
Completing project deliverables is a responsibility of the project coordinator. Usually, failure to complete a deliverable is considered a problem for a EU-funded scientific project and it is ascribed at the project coordinator. However, it should be noted that the regulatory outcome of a clinical trial application is out of the power of the project coordinator and that grant funding body bears no responsibility for that regulatory outcome whereas that outcome is still considered by the grant funding body as a milestone in the project. In addition, timelines of the NCA approval process are frequently not compatible with the timelines of the EU funded projects. Those contradictions need to be generally addressed in order to maximize the usefulness of such research projects for the EU system.

Participation of SME as an industrial partner in EU funded projects is required to add value for European citizens on the output of the project. When the project scope is a clinical trial and the project coordinator is the industrial partner, as in the EURE-CART project, the delay caused by regulatory hurdles might result in a situation where the coordinator has no longer an interest in pushing forward the project, since financial aspects are as much-if not more- important for an industrial partner as compared to scientific aspects. This risk should be considered by applicants when choosing the coordinator of a project that aims at carrying out a clinical trial.

6 | Recommendations for achieving harmonization

Harmonization would be of course obtained when the EU Reg.536 is implemented. In the meanwhile, VHP for GMO-based IMP should still be in place and more robust. The GMO assessment and GMO authorities should be involved in the VHP. Another aspect on which harmonization is required is training of NCA assessors, to avoid situations in which the same body of data is evaluated differently.

7 | Conclusions

All the RAC members and the experts involved in the EURE-CART project agree that a close interaction and knowledge exchange among European NCA, training of assessors and providing applicants with clear requirements and guidance on the application process are crucial for the harmonization of clinical trial approval processes. In any case, the different guidelines available in individual NCA should not hamper clinical trials but help iGTMP development and protect patients. When assessing the deliverables and timelines of projects involving clinical trials, the EU legislation on clinical trials and on GMO should be taken into deeper consideration, if the IMP belongs to the category of GMO/GMMO.

Developing a harmonized and consistent approval process by all of the EU CA (including GMO CA) could significantly mitigate some of the actual gaps present in EU concerning the clinical development of gene therapy medicinal products.

8 | References

1) Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ, 2017, EMBO Molecular Medicine. 9 (9): 1183–1197; S.L.
2) Maude S.L., Laetsch T.W., Buechner J., Rives S., Boyer M., Bittencourt H., Bader P., Verneris M.R., Stefanski H.E., Myers G.D., Qayed M., De Moerloose B., Hiramatsu H., Schlis K., Davis K.L., Martin P.L., Nemecek E.R., Yanik G.A., Peters C., Baruchel A., Boissel N., Mechinaud F., Balduzzi A., Krueger J., June C.H., Levine B.L., Wood P., Taran T., Leung M., Mueller K.T., Zhang Y., Sen K., Lebwohl D., Pulsipher M.A., Grupp S.A., N Engl J Med. 2018 Feb 1; 378(5): 439–448
3) Neelapu S. S., Locke F. L., Bartlett N. L., Lekakis L. J., Miklos D. B., Jacobson C. A., Braunschweig I., Oluwole O. O., Siddiqi T., Lin Y., Timmerman J. M., Stiff P. J., Friedberg J. W., Flinn I. W., Goy A., Hill B. T., Smith M. R., Deol A., Farooq U., McSweeney P., Munoz J., Avivi I., Castro J. E., Westin J. R., Chavez J. C., Ghobadi A., Komanduri K. V., Levy R., Jacobsen E. D., Witzig T. E., Reagan P., Bot A., Rossi J., Navale L., Jiang Y., Aycock J., Elias M., Chang D., Wiezorek J., GoW. Y., 2017, New England Journal of Medicine 377: 2531-2544;
4) M. Wang, Munoz J., Goy A., Locke F.L., Jacobson C.A., Hill Brian T., Timmerman J-M., Holmes H, Jaglowski S., Flinn I.W., McSweeney P.A., Miklos D.B., Pagel J.M., Kersten M.J., Milpied N., Fung H., Topp M.S., Houot R., Beitinjaneh A., Peng W., Zheng L., Rossi J.M., Jain Rajul K., Rao A.V., Reagan P.M., New England Journal of Medicine 382: 1331-1342;
5) Raje N, Berdeja J., Lin Y., Siegel D., Jagannath S., Madduri D., Liedtke M., Rosenblatt J., Maus M.V., Turka A., Lam L.P., Morgan R.A., Friedman K., Massaro M., Wang J., Russotti G., Yang Z., Campbell T., Hege K., Petrocca F., Quigley M.T., Munshi N., Kochenderfer J.N., 2019 New England Journal of Medicine 380: 1726-1737
6) www.ClinicalTrials.gov
7) Legras S., Günthert U., Stauder R., Curt F., Oliferenko S., Kluin-Nelemans H C., Marie J.P., Proctor S., Jasmin C., Smadja-Joffe F. A strong expression of CD44-6v correlates with shorter survival of patients with acute myeloid leukemia. Blood. 1998;91(9):3401-3413.);
8) Liebisch P., Eppinger S., Schopflin C., Stehle G., Munzert G., Dohner H., Schmid M. CD44v6, a target for novel antibody treatment approaches, is frequently expressed in multiple myeloma and associated with deletion of chromosome arm 13q. Haematologica. 2005; 90(4):489-493
9) https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/advanced-therapy-classification
10) https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:en:PDF
11) https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/reg_2014_536_en.pdf
12) https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-studies-required-first-clinical-use-gene-therapy-medicinal-products_en.pdf
13) https://www.ema.europa.eu/en/documents/other/cat-work-plan-2021_en.pdf
14) https://www.hma.eu/ctfg.html;
15) https://www.hma.eu/fileadmin/dateien/Human_Medicines/01About_HMA/Working_Groups/CTFG/2020_10_CTFG_VHP_sponsor_version_5.pdf
16) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009L0041
17) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018
18) https://eur-lex.europa.eu/eli/reg/2004/726/2019-03-30
19) https://ec.europa.eu/health/human-use/advanced-therapies/gmo_investiganional_en
20) https://www.ema.europa.eu/en/documents/other/action-plan-activities-proposed-cat-interested-parties-focus-group-non-clinical-development-advanced_en.pdf

9 | Abbreviations

Abbreviation /acronym	Description
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AML	Acute myeloid leukaemia
ATMP	Advanced Therapy Medicinal Products
CA	Competent Authorities
CAR	Chimeric Antigen Receptor.
CAR-T	Chimeric Antigen Receptor - T cells
CAT	Committee for Advanced Therapies
EC	Ethical Committee
EMA	European Medicines Agency
EU	European Union
EU MS	European Member States
EURE-CART	EURopean Endeavour for Chimeric Antigen Receptor Therapies
GMO/GMMO	Genetically Modified Organisms and Micro-organisms
GTMP	Gene therapy medicinal products
HRS	Ospedale San Raffaele
iGTMP	investigational gene therapy medicinal product
IMPD	Investigational Medicinal Product Dossier
IMP	Investigational medicinal product
MM	Multiple myeloma
ISS	Istituto Superiore di Sanità
NCA	National Competent Authorities
NIBCS-MHRA	National Institute for Biological Standards and Control - Medicines and Healthcare products Regulatory Agency
OPBG	Ospedale Pediatrico Bambin Gesù
PAU	Hospital de la santa creu i sant pau (PAU)
RAC	Regulatory Advisory Committee
UHO	University Hospital Ostrava
UKW	University Hospital Würzburg
VHP	Voluntary Harmonization Procedure
VVKT	Valstybinė vaistų kontrolės tarnyba
WG	Working Group
WP3	Work Package 3 of EURE-CART Project « Regulatory approval of the EURE-CART cell product »