



# Supplementary Materials

## Extracellular vesicles as next generation diagnostics and advanced therapy medicinal products

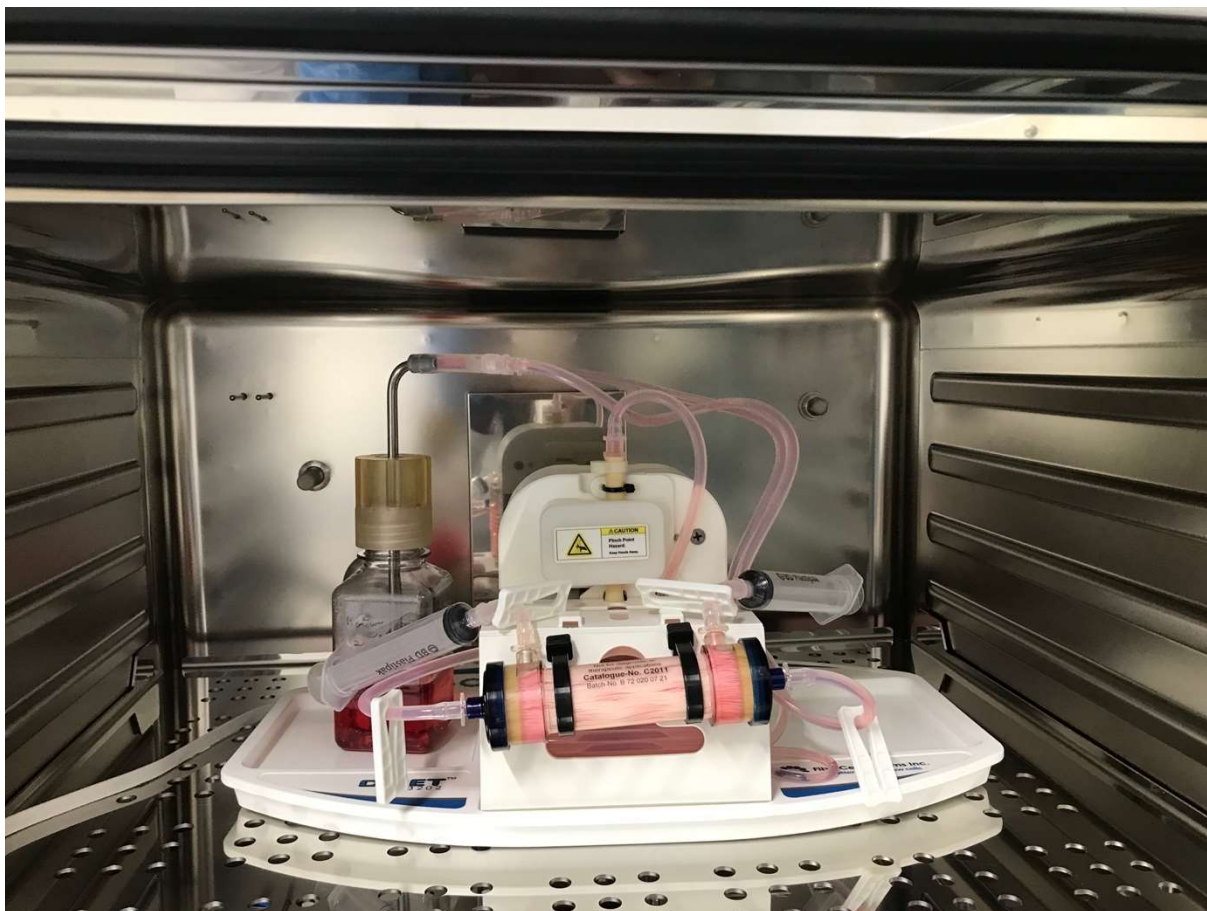
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**Figure S1.** A photo of the hollow fiber bioreactor (HFB). The media reservoir, peristaltic pump, and an extralong silicone tube, acting as an oxygenator, are connected to the intracellular space of the HFB cartridge. The HFB system is operated at the Cell and Tissue Laboratory of the Department of Toxicology and Food Science, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland.



**Table S1.** The legal requirements and principle regulatory guidelines to be taken into consideration during the pharmaceutical development, non-clinical studies and clinical trials of EV-based medicinal products.

| Reference number                            | Guideline title   | Effective date   |
|---|---|------------------|
| <b>Pharmaceutical development / quality</b> |   |                  |
| ICH Q2(R2)                                  | Validation of analytical procedures   | 1 November 2023  |
| ICH Q5A(R2)<br>EMA/CHMP/ICH/804363/2022     | Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin                                    | 14 June 2024     |
| ICH Q5C                                     | Quality of biotechnological products: stability testing of biotechnological/ biological products  | 30 November 1995 |
| ICH Q5D                                     | Derivation and characterisation of cell substrates used for production of biotechnological/ biological products                                     | 16 July 1997     |
| ICH Q5E                                     | Comparability of biotechnological/ biological products subject to changes in their manufacturing process  | 18 November 2004 |
| ICH Q6B<br>CPMP/ICH/365/96                  | Specifications: test procedures and acceptance criteria for biotechnological/ biological products   | 1 September 1999 |
| ICH Q7                                      | Good manufacturing practice guide for active pharmaceutical ingredients   | 10 November 2000 |
| ICH Q9(R1)                                  | Quality risk management   | 18 January 2023  |
| EMA/CHMP/BWP/706271/2010                    | Plasma-derived medicinal products   | 1 February 2012  |
| EMA/CHMP/410869/2006 (*)                    | Human cell-based medicinal products   | 1 September 2008 |
| EMA/CAT/571134/2009 (*)                     | Stem cell-based medicinal products  | 14 January 2011  |
| CPMP/BWP/1143/00                            | Position statement on the use of tumorigenic cells of human origin for the production of biological and biotechnological medicinal products         | February 2001    |
| EMA/CHMP/BWP/187338/2014                    | Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission | 1 November 2016  |



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|---------------------------------|---|---|
| 3ab1aen                         | Production and quality control of medicinal products derived by recombinant DNA technology  | 1 July 1995   |
| EMA/CHMP/BWP/729106/2011        | Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products   | 1 December 2013   |
| 3ab6a (*)                       | Gene therapy product quality aspects in the production of vectors and genetically modified somatic cells  | 1 July 1995   |
| EMA/CAT/499821/2019 (*)         | Questions and answers on comparability considerations for advanced therapy medicinal products (ATMP)  | Adopted on 6 December 2019  |
| EMA/410/01 Rev. 3               | Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products  | 1 July 2011   |
| GMP annex 13                    | Manufacture of Investigational Medicinal Products   | 31 July 2010  |
| GMP annex 13                    | Detailed Commission guideline of 8 December 2017 on the good manufacturing practice for investigational medicinal products pursuant to the second paragraph of the Article 63(1) of Regulation (EU) No 536/2014 | The Regulation 536/2014 is fully in force since 31 January 2023           |
| EMA/CHMP/BWP/534898/2008 Rev. 2 | Requirements for quality documentation concerning biological investigational medicinal products in clinical trials  | 31 January 2022   |
| EMA/CHMP/BWP/398498/05          | Virus safety evaluation of biotechnological investigational medicinal products  | 1 February 2009   |
| EMA/CAT/852602/2018 (*)         | Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials   | Draft adopted in January 2019   |
| ICH M4 (Quality dossier)        | Common Technical Document   | November 2000   |
| <b>Non-clinical development</b> |   |   |
| ICH S6 (R1)                     | Preclinical safety evaluation of biotechnology-derived pharmaceuticals  | Parent Guideline dated 16 July 1997<br>Addendum incorporated in June 2011 |



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| EMA/CHMP/SWP/28367/07 Rev. 1  | Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products | 01 February 2018        |
| ICH S9  | Non-clinical evaluation for anticancer pharmaceuticals  | 29 October 2009         |
| EMA/CHMP/GTWP/125459/2006 (*)   | Non-clinical studies required before first clinical use of gene therapy medicinal products  | November 2008           |
| ICH M3 (R2)   | Guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals            | 11 June 2009            |
| ICH S2 (R1)   | Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use   | 9 November 2011         |
| CPMP/SWP/1042/99 Rev 1 Correction   | Guideline on repeated dose toxicity   | 01 September 2010       |
| ICH S3A   | Note for guidance on toxicokinetics: the assessment of systemic exposure in toxicity studies  | 27 October 1994         |
| ICH S4<br>CPMP/ICH/300/95   | Duration of chronic toxicity testing in animals (rodent and non-rodent toxicity testing)  | 1 May 1999              |
| ICH S3B   | Pharmacokinetics: Guidance for repeated dose tissue distribution studies  | June 1995               |
| ICH S7A   | Safety pharmacology studies for human pharmaceuticals   | 8 November 2000         |
| ICH S7B   | The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals     | 12 May 2005             |
| ICH S12 (*)   | Non-clinical biodistribution considerations for gene therapy products   | 14 March 2023           |
| ICH M10   | Bioanalytical method validation and study sample analysis   | 24 May 2022             |
| EMA/CHMP/SWP/169215/2005  | Need for non-clinical testing in juvenile animals on human pharmaceuticals for paediatric indications                                       | 01 August 2008          |
| ICH M4 (Non-clinical dossier)   | Common Technical Document   | November 2000           |
| <b>Clinical development</b> ( <i>the scope of guidelines depends vastly on the planned indication</i> ) |   |                         |
| Regulation (EU) No 536/2014   | Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for     | Adopted on 16 June 2014 |



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|  | human use, and repealing Directive 2001/20/EC   | Fully in force since 31 January 2023 |
| ICH E6(R2)   | Integrated addendum to ICH E6(R1): guideline for good clinical practice   | 9 November 2016                      |
| ICH E8(R1)   | General considerations for clinical studies   | 6 October 2021                       |
| ICH E9   | Statistical principles for clinical trials  | 5 February 1998                      |
| ICH E1<br>CPMP/ICH/375/95  | Population exposure: The extent of population exposure to assess clinical safety  | June 1995                            |
| ICH E10  | Choice of control group and related issues in clinical trials   | 20 July 2000                         |
| E2A-E2F  | Pharmacovigilance   | 1994 - 2012                          |
| ICH E4   | Dose-response information to support drug registration  | 10 March 1994                        |
| ICH E3   | Structure and content of clinical study reports   | 30 November 1995                     |
| ICH E16  | Biomarkers related to drug or biotechnology product development: context, structure and format of qualification submissions | 20 August 2010                       |
| E18  | Guideline on genomic sampling and management of genomic data  | 3 August 2017                        |
| EMA/CHMP/EWP/192217/2009<br>Rev.1 Corr.2**                         | Bioanalytical method validation   | 1 February 2012                      |
| 3CC3a  | Pharmacokinetic studies in man  | 1 October 2015                       |
| EMA/CHMP/205/95 Rev.6  | Guideline on the clinical evaluation of anticancer medicinal products   | Adopted on 18 November 2023          |
| CHMP/EWP/83561/2005  | Guideline on clinical trials in small populations   | 1 February 2007                      |
| EMA/149995/2008 (*)  | Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products                       | 1 January 2009                       |
| ICH M4 (Clinical dossier)  | Common Technical Document   | November 2000                        |
| <b>General requirements for ATMP, which may be adopted for EVs</b> |   |                                      |
| Regulation EC/1394/2007  | Regulation on advanced therapy medicinal products.  | 30 December 2007                     |



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| EMA/CAT/600280/2010 Rev. 1 | Reflection paper on classification of advanced therapy medicinal products  | Adopted on 22 May 2015      |
| EMA/CAT/CPWP/686637/2011   | Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products. | 12 February 2013            |
| EMA/CAT/80183/2014         | Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products   | Draft adopted in March 2018 |