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Primary Chondroprogenitors: Standardized & Versatile Allogeneic Cytotherapeutics

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Definition: Primary chondroprogenitors obtained from standardized cell sources (e.g., FE002 clinical grade cell sources) may be cultured in vitro and may be cytotherapeutically applied in allogeneic musculoskeletal regenerative medicine. Multicentric translational research on FE002 human primary chondroprogenitors under the Swiss progenitor cell transplantation program has notably validated their robustness and high versatility for therapeutic formulation in clinically compatible prototypes, as well as a good safety profile in diverse in vivo preclinical models. Therein, stringently controlled primary cell source establishment and extensive cell manufacturing optimization have technically confirmed the adequation of FE002 primary chondroprogenitors with standard industrial biotechnology workflows for consistent diploid cell biobanking under GMP. Laboratory characterization studies and extensive qualification work on FE002 progenitor cell sources have elucidated the key and critical attributes of the cellular materials of interest for potential and diversified human cytotherapeutic uses. Multiple formulation studies (i.e., hydrogel-based standardized transplants, polymeric-scaffold-based tissue engineering products) have shown the high versatility of FE002 primary chondroprogenitors, for the obtention of functional allogeneic cytotherapeutics. Multiple in vivo preclinical studies (e.g., rodent models, GLP goat model) have robustly documented the safety of FE002 primary chondroprogenitors following implantation. Clinically, FE002 primary chondroprogenitors may potentially be used in various forms for volumetric tissue replacement (e.g., treatment of large chondral/osteochondral defects of the knee) or for the local management of chondral affections and pathologies (i.e., injection use in mild to moderate osteoarthritis cases). Overall, standardized FE002 primary chondroprogenitors as investigated under the Swiss progenitor cell transplantation program were shown to constitute tangible contenders in novel human musculoskeletal regenerative medicine approaches, for versatile and safe allogeneic clinical cytotherapeutic management.

Keywords: bioengineering; chondral/osteochondral defects; chondrogenesis; cytotherapeutics; formulation; musculoskeletal pathologies; primary FE002 chondroprogenitors; regenerative medicine; safety; translational research

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1. Introduction

Musculoskeletal diseases in general and chondral/osteochondral affections, in particular, are highly incident in aging patient populations [1–4]. While conservative orthopedic best practices enable the successful clinical management of critical cases of cartilage injury or degeneration (e.g., prosthetic replacement), effective regenerative medicine interventions and solutions are necessary in the cases of moderate to severe affections [3,5–8]. Therefore, many natural and artificial biomaterials or bioengineered constructs have been successfully clinically applied for chondropathies and cartilage tissue defects, with extensive available hindsight (i.e., intervention safety, quality, efficacy) [5,9–18]. Parallelly, important translational efforts, deployed over the past 40 years, have led to the implementation of diverse clinical protocols for several generations of autologous chondrocyte implantation (ACI) [1,4,8,19–28]. While initial and successful approaches to ACI may have relied on the use of cultured cells or minimally manipulated chondrocyte suspensions, current commercially available clinical approaches to cartilage regenerative medicine often comprise the use of a matrix/scaffold component (i.e., combination products, e.g., cells in a hyaluronan-based hydrogel scaffold or bilayer collagen constructs) [1,10,11,28–37].

Vast arrays of potential cell sources (e.g., various stem and progenitor cells, somatic cells, platelets, etc.) and processing methods (e.g., preparation of cell suspensions, spheroids) have been investigated for the high-quality cytotherapeutic management of chondropathies and chondral/osteochondral defects [7,11,38–42]. Recently, multiple genetically modified cell lines, designed for enhanced chondrogenic function, have been studied and clinically proposed for cartilage tissue engineering [6,43–45]. From a technical standpoint, the scientific knowledge of the in vitro behavior and functional evolution (i.e., transiently reduced chondrogenesis potential in monolayer cellular expansion) of cultured chondrocytes has rapidly increased [5,8,14,31,46–55]. For therapeutic cell manufacturing purposes, numerous studies have enabled and have validated (i.e., from technical, quality, and functional standpoints) the substitution of fetal bovine serum (FBS) by human platelet lysates (HPL) as cellular growth medium supplements [28,56–63]. Notwithstanding, despite enormous progress in the biotechnological and bioengineering approaches to cell-based combination products for cartilage repair and regeneration, important regulatory and clinical bottlenecks have recently been documented [28,40,64–70]. Indeed, specific quality-oriented and process-based approaches to cell therapy manufacturing have become the norm (e.g., application of cGMPs for cellular active substances and finished cell-based product manufacture) [28]. Importantly from the clinical standpoint, the cartilage lesion localization, the surgical approach, and the patient follow-up management plan have been identified as critical factors for consistently attaining long-term clinical success with cytotherapies for cartilage tissue affections [14,65,69].

Human primary chondroprogenitors (e.g., FE002 clinical grade cell sources) have been extensively investigated under the Swiss progenitor cell transplantation program as a potential cytotherapeutic solution for the optimal homologous allogeneic management of diverse cartilage tissue disorders [38,71,72]. Human FE002 primary chondroprogenitors are cultured diploid cells, inherently pre-terminally differentiated, which display monomodal and stable phenotypes in vitro [38,72]. Homogeneous and robust cryopreserved cell banks and cell lots of FE002 primary chondroprogenitors may be exploited as highly sustainable tools and material sources for allogeneic musculoskeletal cytotherapeutic applications under modern restrictive quality requirements [71,72]. Importantly, human FE002 primary chondroprogenitors are highly biocompatible with diverse biomaterials, possess an inherent immune privilege, and present no known tumorigenic behaviors [38,72].

Such standardized biological materials are biotechnologically manufactured and are formulated following best practices in pharmaceutical sciences and cell-based bioengineering, with the central therapeutic objectives of rapidly and optimally restoring chondral tissular structures and functions [72]. Overall, the FE002 primary chondroprogenitors investigated under the Swiss progenitor cell transplantation program were shown to present high robustness and versatility in an array of potential therapeutic uses (e.g., fresh or off-the-

freezer cell therapies) in human musculoskeletal regenerative medicine [71,72]. A succinct overview of the currently published body of knowledge (i.e., scientific peer-reviewed elements) on FE002 primary chondroprogenitors is presented in Table 1.

Table 1. Summary of the published peer-reviewed reports describing the collaborative and multicentric translational work (i.e., characterization, qualification, validation) on FE002 primary chondroprogenitors under the Swiss progenitor cell transplantation program. This constantly evolving body of knowledge has established FE002 primary chondroprogenitors as standardized and versatile cytotherapeutic contenders for human musculoskeletal regenerative medicine, for repair promotion and/or regeneration support in chondral/osteochondral affections. CAM, chorioallantoic membrane model; GLP, good laboratory practices; HA, hyaluronic acid.

Study Subject/Domain	Scope of the Study/Investigated Parameters/Main Data	References
1. Progenitor Cell Source Establishment	Biological starting material procurement (i.e., controlled organ donation within the Swiss progenitor cell transplantation program) and establishment of FE002 primary progenitor cell sources in a cryogenically preserved multi-tiered cell bank system.	[71]
2. In Vitro Cell Type Characterization	Characterization of progenitor cell type key and critical attributes (e.g., cellular proliferative behavior in culture, cellular lot homogeneity and purity, cell genetic and phenotypic stability, proteomics, chondrogenic potential, in vitro safety parameters).	[38,72]
3. Characterization of In Vitro Mechanobiological Cellular Behavior	Study of the influence of physical (i.e., mechanical) parameters on cellular biology and functional attributes [1]. Optimization of physical processing workflows for cytotherapeutic material lots.	[73–75]
4. In Vitro Cell Banking & Biotechnological Manufacturing	Optimization and standardization of in vitro progenitor cell manufacturing workflows (i.e., industrial-scale cellular lots). Confirmation of progenitor cell source sustainability at passage levels for clinical use [2].	[72]
5. Formulation Studies for Functional Cytotherapeutic Products	Formulation and translational characterization/qualification of hydrogel-based (e.g., modified HA-based gels) standardized transplants and polymeric scaffold-based tissue engineering products yielding viable/functional progenitor cells.	[76–80]
6. In Vivo Preclinical Safety Assessments	Study of progenitor cellular material or cytotherapeutic combination product safety in ovo (i.e., standardized CAM model) and in vivo (e.g., subcutaneous rodent implantation models, GLP study of knee chondral defect management in goats).	[72,76,77,79]

[1] It is noteworthy that the considered tissue engineering products/prototypes were reported to be characterized by endpoint mechanical attributes which did not match those of native chondral tissues. This aspect has not been interpreted negatively, based on the fact that such orthopedic cell-based approaches aim to stimulate repair and/or support regeneration processes, rather than exclusively structurally replacing the damaged cartilage. Therefore, while the implanted constructs must be able to bare weight, sufficient potential for mechanical adaptation to the local healing environment must remain, for optimal graft integration and therapeutic deployment of functional attributes. [2] The established models have outlined that a single clinical grade primary chondroprogenitor cell source could potentially yield several million therapeutic bioengineered cartilage grafts or injectable viable cell suspensions, without the need for repetition of the cell type establishment phase.

Notably, multiple in vivo preclinical studies (e.g., in rodent and goat models) have robustly documented the safety of FE002 primary chondroprogenitors following implantation, which may therefore be safely considered for investigational human cytotherapeutic use (i.e., international first-in-man clinical trials) [72,76,77,79]. From a clinical indication standpoint, such cellular materials and combinations thereof may potentially be used for volumetric tissue replacement (e.g., treatment of extensive chondral/osteochondral defects of the knee) or the local management of mild to moderate chondral affections and pathologies (i.e., injectable hydrogels in osteoarthritis patients) [72]. Overall, the aggregated multicentric translational work on FE002 primary

progenitor cell sources, performed over the past decade in Switzerland, has confirmed their high versatility and safety for application as cellular active ingredients within the development of novel cytotherapeutic products and standardized transplants for human use (Table 1) [38,72,76–80].

2. Primary Chondroprogenitors for Novel Allogeneic Tissue Engineering Applications: High International Focus & Published Translational Studies

Primary chondroprogenitors initially derived from prenatal tissues are often mentioned by researchers investigating potentially optimal therapeutic primary cell sources for allogeneic cartilage tissue engineering [3,14,38,76]. In addition to the high robustness and stability of these non-modified biological materials (i.e., "extensively manipulated" by cellular expansion, but without gene editing), the reported maintenance of chondrogenic functional potential by such cultured cells is critical for tissue engineering product function/efficacy [38,72]. Furthermore, the immune privilege of such primary progenitor cell sources procures tangible advantages in an allogeneic or xenogeneic therapeutic setting, for avoiding rejection by an immunocompetent host/recipient [71,72].

The scope of the present entry was set on the FE002 primary chondroprogenitors studied under the Swiss progenitor cell transplantation program, as all the considered cellular materials were derived from the same initial organ donation (i.e., FE002 donation, Table 1) [71,72]. However, important parallel preclinical work by international research groups has also focused on similar processes or uses of (i.e., primary or transformed) prenatal cartilage-derived progenitor cells and is noteworthy. Translational work on such primary cells has notably been undertaken by Korean- and US-based groups, reaching advanced preclinical model levels [81–90]. Specifically, the applicability and functionality of clinically compatible bioengineered prototypes containing primary chondroprogenitors were shown in vivo [81,83,84]. Therein, similar conclusions were drawn (i.e., as compared to the various Swiss groups) about the vast potential of the considered biological materials for high-quality allogeneic tissue engineering and alternative clinical applications in human orthopedic medicine [81–83,87].

Furthermore, a most notable and unique landmark in cell and gene therapies for cartilage tissue engineering is the historical case of Invossa (Tonogenchoncel-L, TissueGene-C, Kolon TissueGene, Rockville, MD, USA), a first-in-class orthopedic cell and gene therapy product [45]. Initially studied at preclinical levels over the 2000–2010 decade, this preparation comprises a mixture of cell populations [91–94]. Indeed, Invossa is reported to contain allogeneic non-transformed cells (i.e., chondrocytes from human polydactyly tissue) and retrovirally transduced human chondrocytes expressing TGF-β1 for enhanced functionality [95]. However, of particular interest, Invossa was instead reported to contain HEK-293 cells (i.e., immortalized cell line, derived from human embryonic kidney tissue) [45,96]. Therefore, instead of the transduced chondrocytes as initially described for Invossa, it was HEK-293 cells expressing TGF-β1 that were mixed with the allogeneic chondrocytes. Despite thunderous clinical trial interruptions following this gross mislabeling or cellular cross-contamination event, the clinical safety of the intervention (i.e., absence of adverse event occurrence, meeting of efficacy endpoints) was documented and enabled promoters to obtain regulatory approvals to continue the investigative human clinical work in the USA [45,96]. Of utmost importance, the fact that several clinical stages were successively and successfully passed by Invossa over the 2010–2020 decade and its ongoing investigational use (i.e., multi-centric clinical trials) in the USA create a strong and undeniable precedent for the direct use of prenatal tissue-derived cytotherapeutics (e.g., HEK-293 cells) in human regenerative medicine [6,97,98]. Specifically, while several technical elements and deviations remain to be clarified in the case of Invossa (i.e., potential cross-contaminations by HEK-293 stocks during therapeutic cell manufacturing), the specified orthopedic clinical endpoints were reported to have been met [45].

3. Starting Biological Material Procurement & Clinical-Grade FE002 Primary Chondroprogenitor Cell Source Establishment Methodology

Modern quality, safety, and traceability requirements around the processing of cytotherapeutic materials for human investigational regenerative medicine use are currently stringent and necessitate extensive methodological planning and documentation [99]. Therein, good manufacturing practices (GMP) must be employed notably for combined advanced therapy medicinal products (cATMP), which encompass most bioengineered cartilage tissue grafts requiring cell culture steps (i.e., substantial manipulation of the cells) [72]. Furthermore, close consideration of the locally applicable legal and ethical framework is necessary for biological starting material (i.e., cartilage tissue) procurement and for subsequent clinical grade progenitor cell source establishment [71,99]. In particular, up-to-date legal and ethical guidelines must constitute the basis of the methodological devising of starting material procurement and bioprocessing for clinical cytotechnologies and cytotherapeutics [71]. These elements are key in view of demonstrating to national regulators and health authorities that specific primary progenitor cell sources are fit for human investigational clinical use. Therefore, the considered examples of FE002 primary progenitor cell sources were established in Switzerland under a validated progenitor cell transplantation program, with standardized whole-cell bioprocessing and cell banking in view of eventual clinical and commercial use (Figure 1) [71].

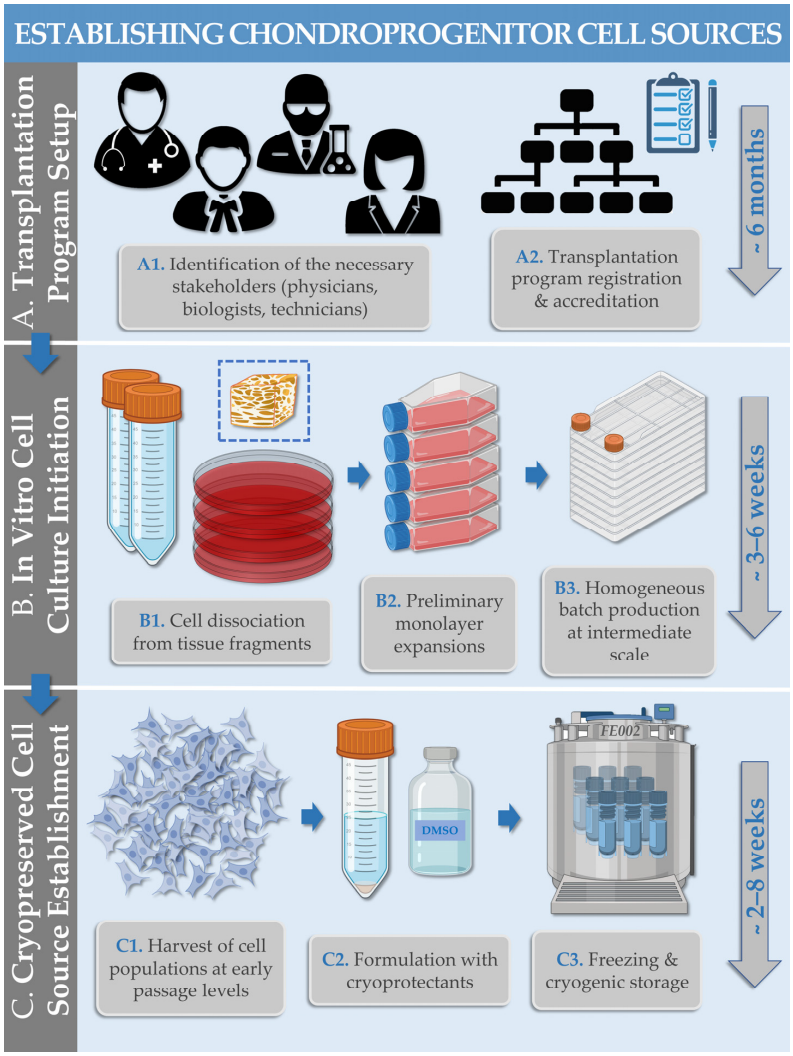

Figure 1. Schematic and illustrated methodological workflow describing the establishment of FE002 primary progenitor cell sources under the Swiss progenitor cell transplantation program. (**A1**,**A2**)

Appropriate roles and responsibility attribution within the operational team (i.e., for starting material procurement) is critical for the methodological soundness and accreditation of the cell transplantation program. (**B1**–**B3**) Following rapid initial cellular isolation from starting materials, serial in vitro cellular expansions are performed during the adherent cell culture initiation phase. (**C1**–**C3**) The obtained cellular materials are harvested after the monolayer expansion and are appropriately processed for cryopreservation in a cell bank system. Approximative timelines for individual phase execution are presented in blue font. DMSO, dimethyl sulfoxide.

Importantly, the robustness of primary progenitor cell biobanking and subsequent biotechnological cell manufacturing processes is a key technical parameter for obtaining homogeneous, high-quality, and consistent cellular harvests [71,72]. This enables to sustainably exploit defined progenitor cell sources and to perform extensive qualification/validation work on the manufacturing process and the obtained material lots, contrasting with autologous cell sources (i.e., usually highly limited in material availability) used in ACI [28,72]. Ultimately, these elements are useful and necessary for demonstrating the technological soundness and the preclinical quality of the retained allogeneic cytotherapeutic approach.

4. Optimized & Standardized FE002 Primary Chondroprogenitor Cell Banking & Biotechnological Manufacturing Processes

As previously mentioned for primary progenitor cell source establishment, the use of robust processes is essential for maintaining both the high quality and the sustainability of the considered biological source [72]. Therefore, process optimization studies and standardization work are critical early elements of industrial manufacturing process development and validation, enabling the specification of key and critical process parameters (KPP, CPP) or attributes [28]. Starting with seeding cell lots (i.e., usually parental cell bank (PCB) materials), all of the in vitro manufacturing activities are carried out according to conservative best practices of primary diploid cell culture (e.g., serial multi-tiered cell banking and cryopreserved vial lot manufacture, Figure 2) in grade A GMP manufacturing environments [72].

Due to the documented high cellular robustness and overall in vitro stability of the studied FE002 chondroprogenitors, consistent technical specifications (e.g., cell seeding and harvest densities, culture medium exchange intervals, total cell culture periods) may be used for the successive adherent cellular expansion rounds of manufacturing campaigns (Table 1, Figure 3) [72].

Once the progenitor cell lot manufacturing activities are performed (i.e., including GMP-specific in-process controls, IPC), appropriate safety and quality-oriented post-process controls (PPC, i.e., characterization or qualification assays, release testing) are performed on the cryopreserved vial lots [28,71]. FE002 progenitor cellular materials for human therapeutic use are usually manufactured in a GMP cell bank system, where biosafety testing schemes are adapted from Ph. Eur. general chapter 5.2.3 and ICH Q5A (Table 2) [100,101].

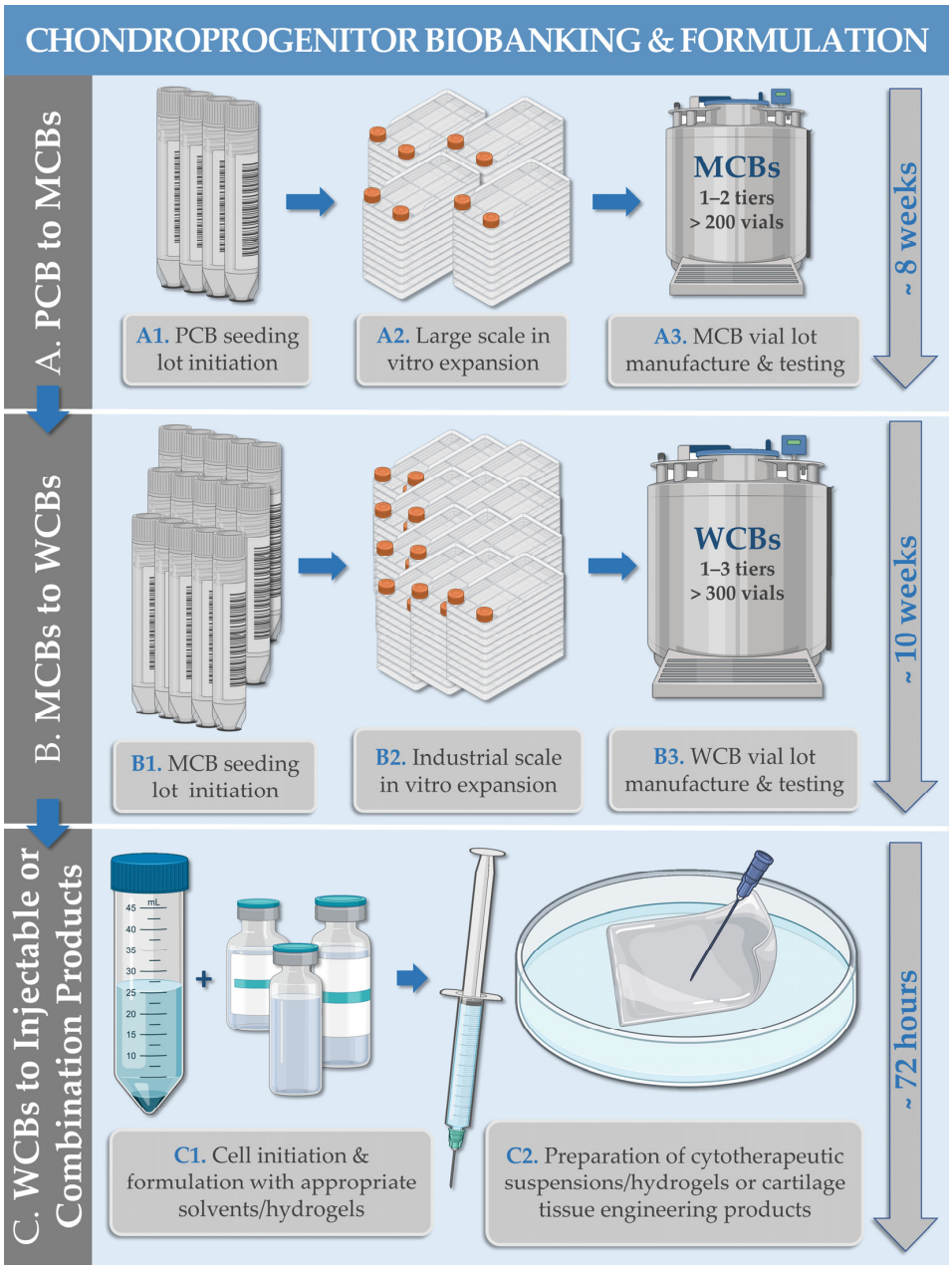

Figure 2. Schematic and illustrated methodological workflow describing the multi-tiered cell banking and some of the combined cytotherapeutic uses of primary chondroprogenitors (e.g., FE002 primary progenitor cell sources). (**A1**–**A3**) Parental cell bank (PCB) cellular materials (i.e., cells at early in vitro passage levels) are used to generate master cell bank (MCB) materials (i.e., cells at intermediate in vitro passage levels). (**B1**–**B3**) MCB cellular materials are used to generate working cell bank (WCB) materials (i.e., cells at in vitro passage levels appropriate for clinical use). (**C1**,**C2**) WCB cellular materials may eventually be used for off-the-freezer reconstitution of injectable combination products (e.g., cell suspensions in autologous serum-based solutions or hyaluronan-based hydrogels) or for seeding of bioengineered cartilage grafts (e.g., use of Chondro-Gide collagen scaffolds). Within GMP-compliant multi-tiered biobanking systems, the various cell bank tiers are characterized by the in vitro cell passage level or by the in vitro cell population doubling level of the corresponding cellular population, in the sequential order of increasing levels along the progenitor cell manufacturing workflow. Approximative timelines for individual phase execution are presented in blue font. GMP, good manufacturing practices; MCB, master cell bank; PCB, parental cell bank; WCB, working cell bank.

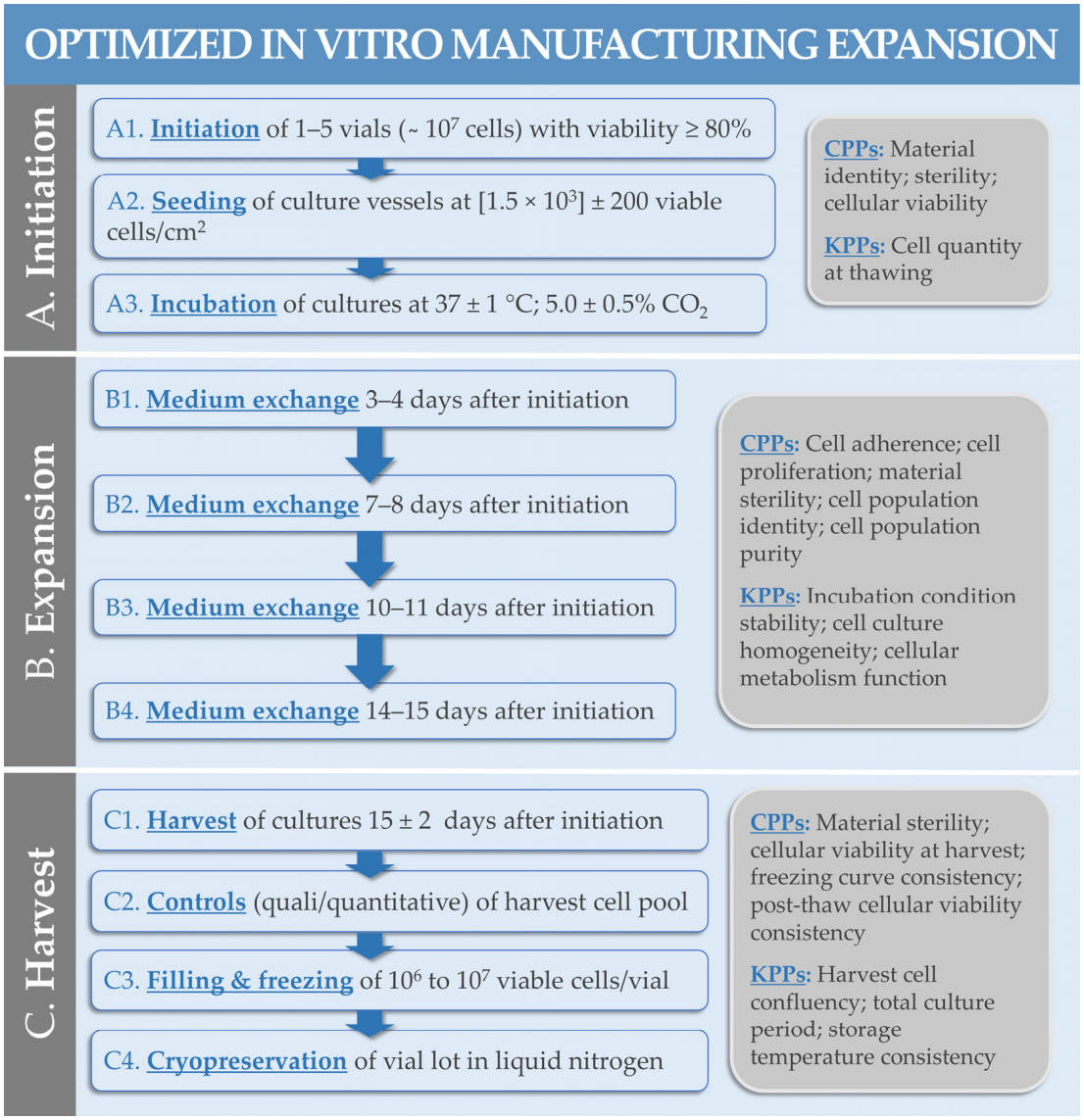

Figure 3. Schematic methodological workflow describing an entire optimized in vitro expansion process for primary chondroprogenitors under the Swiss progenitor cell transplantation program (e.g., FE002 primary progenitor cell sources). A single cellular expansion phase is used within the defined cell banking workflow to sequentially and incrementally generate cell lots of superior passage levels. Consistent technical specifications (e.g., cell seeding densities, harvest cell confluency levels) are used for the in vitro cellular expansions throughout the entire progenitor cell biobanking workflow. All contact-process consumables (e.g., cell culture vessels), materials, and reagents (e.g., culture medium nutritive supplement, cell dissociation reagent, cell cryopreservation solution) are qualified/validated before production and all equipment or techniques are validated. (**A1**–**A3**) Initiation of the seeding cellular materials is performed by retrieval, controlled thawing and assessment, and distribution of the cells in the retained cell culture system. (**B1**–**B4**) The cellular expansion phase comprises incubation (i.e., 37 °C; 5% CO_2) of the cell cultures and periodic cell culture medium exchange procedures. The nutritive component of the cell proliferation medium is classically 10% *v*/*v* FBS. (**C1**–**C4**) Endpoint enzymatic harvest of the expanded cell population is performed and enables the constitution of a new cell bank lot, following conditioning for storage and controlled-rate freezing of the bulk cellular suspension. Possible CPPs and KPPs are specified for individual process phases. CPP, critical process parameter; FBS, fetal bovine serum; KPP, key process parameter.

Table 2. Generic testing requirements for clinical grade FE002 progenitor cytotherapeutic materials (i.e., primary diploid cell type) within a GMP multi-tiered cell bank system, adapted from Ph. Eur. general chapter 5.2.3. The implementation of specific tests and assays at specific stages/tiers of the cell banking process is designed and performed by the manufacturer, based on specific and appropriate risk analyses. EOPCB, end-of-production cell bank; GMP, good manufacturing practices; MCB, master cell bank; PCB, parental cell bank; WCB, working cell bank.

Testing Type/Assay Type	Testing Class/Testing Purpose	Testing Tiers [1]
1. Morphology & proliferative behavior	Identification/general qualification	PCB; MCB; WCB; EOPCB [2]
2. Cell type identification & fingerprinting	Identification/general qualification	PCB; MCB; WCB; EOPCB
3. Cell type karyotype	Identification/general qualification	PCB; MCB; EOPCB
4. Cell type in vitro lifespan	Identification/general qualification	MCB; WCB
5. Testing for bacterial & fungal agents	Extraneous agent detection/microbiological qualification	MCB; WCB
6. Testing for mycobacteria and mycoplasmas	Extraneous agent detection/microbiological qualification	MCB; WCB
7. Testing for viruses and for retroviruses	Extraneous agent detection/microbiological qualification	EOPCB
8. Electron microscopy characterization	Identification/general qualification/extraneous agent detection/microbiological qualification	EOPCB
9. Safety/toxicity testing in ovo or in small animals	Microbiological qualification/safety qualification	EOPCB
10. Tumorigenicity assays	Safety qualification	EOPCB

[1] For various assays, testing is performed on materials characterized by an in vitro passage level $\geq$ to that useful for production purposes, for sparing use of cells at lower passage levels. Distinctions are made between characterization testing and release testing of cell bank lots, where the level of testing depends on the risk analysis. [2] An EOPCB is composed of cellular materials at in vitro passage levels > to that useful for WCB lots and production purposes.

Despite the existence of minimal standards and basic requirements, each cytotherapeutic product manufacturing process is different (i.e., "the process is the product") and must be considered from an overall quality and safety point-of-view [99]. Therefore, both the frequency and the extent of the necessary testing are based on justified risk analyses performed by the manufacturer (Table 2). From a technical standpoint, the use of defined-composition cell proliferation media or cell cryopreservation media is in accordance with modern guidelines, yet the highest attention must be paid to benchmarking and stringent qualification/validation of such elements against conservative standards (e.g., FBS, DMSO-based cryoprotectant solutions) as concerns stability and functional parameters (i.e., ICH Q5E, Q11) [72]. Finally, preclinical research and early process validation steps should be undertaken with manufacturing scalability and GMP-transposition objectives in mind, to rationalize the fixed costs of product/protocol development [71].

5. FE002 Primary Chondroprogenitor In Vitro Characterization & Qualification Data: Robust Fibroblastic Cells with Conserved Chondrogenic Functions

Multicentric characterization and qualification studies, collaboratively performed in Switzerland on FE002 primary chondroprogenitors, have been enabled to gather substantial data in view of their further translational use and clinical applications (Table 1). Notably, complementary research groups from two Universities and two federal polytechnical schools (i.e., Lausanne and Zurich, respectively) have locally contributed to enhancing the multifaceted body of knowledge existing around such potential cytotherapeutic materials (Table 1, Figure 4).

Overall, the reported scientific and technical body of knowledge around the considered FE002 primary chondroprogenitors was found to be in line with alternative literature reports as concerns the applicability of such cellular materials in therapeutic cartilage bioengineering [72,81,83,84]. Specifically, the accumulated data has underscored that such biological materials were well-adapted for the standardized manufacturing of novel allogeneic cytotherapies for human clinical use (Table 1) [72]. Therein, high versatility

in the formulation options for the cellular active substance of interest was confirmed, as detailed hereafter.

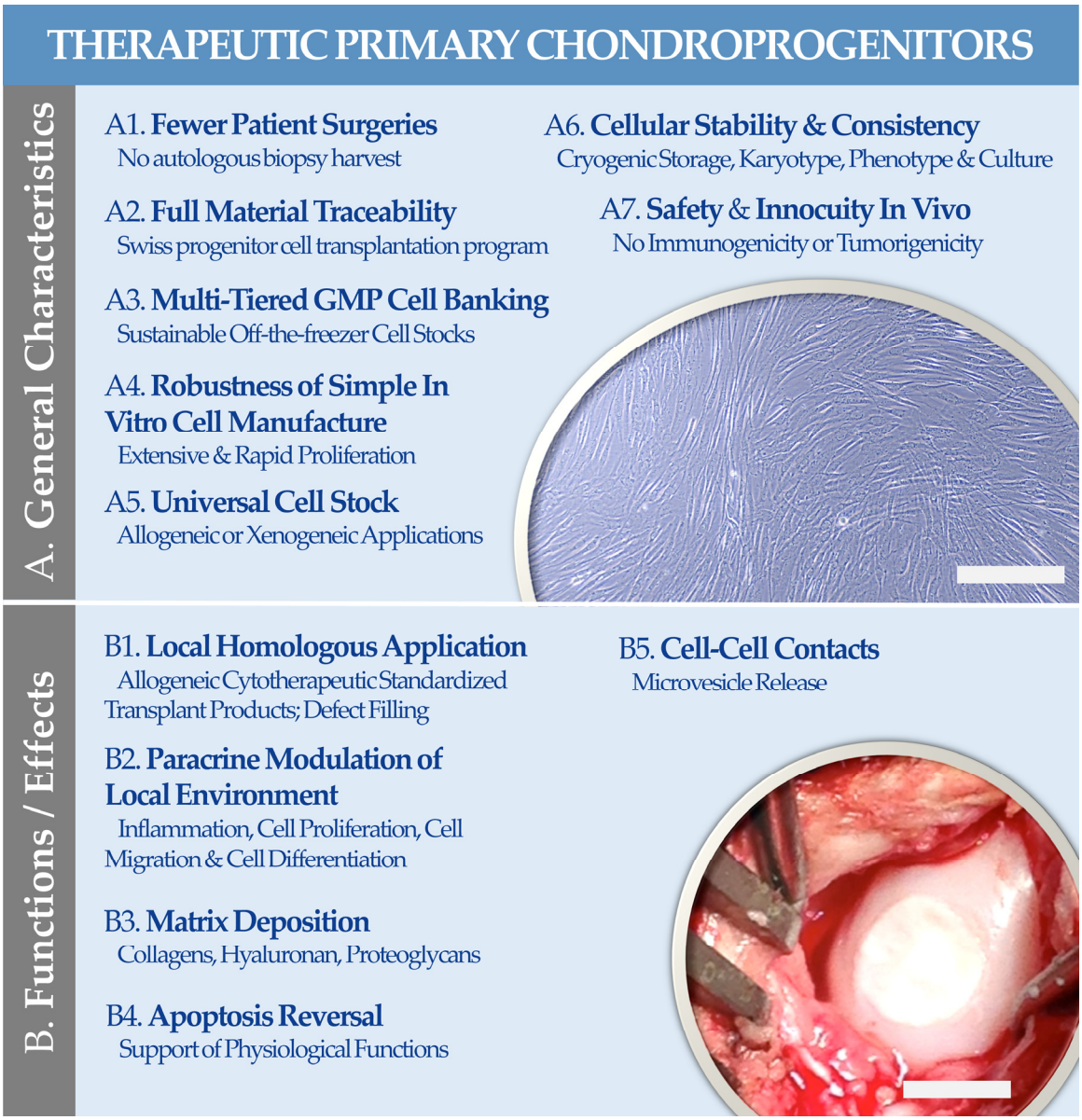

Figure 4. An illustrated summary of the (**A1**–**A7**) general characteristics and of the (**B1**–**B5**) possible functions or effects of FE002 primary chondroprogenitors for therapeutic use in allogeneic orthopedic regenerative medicine. (**A**) The illustration represents contrast phase microscopic records of adherent FE002 chondroprogenitors at the end of the in vitro proliferation phase. Scale bar = 100 µm. (**B**) The illustration represents a bioengineered cartilage graft, consisting of FE002 primary chondroprogenitors seeded on a bilayer collagen scaffold (Chondro-Gide, Geistlich Pharma, Wolhusen, Switzerland), following implantation in the knee joint in a GLP goat model of chondral defect. Scale bar = 7.5 mm. GLP, good laboratory practices; GMP, good manufacturing practices. Modified and adapted from [72].

6. Therapeutic Formulation Options for FE002 Primary Chondroprogenitors: High Versatility in Potential Cell-Assisted Orthopedic Applications

The multicentric formulation studies performed in Switzerland using FE002 primary chondroprogenitors for orthopedic standardized transplant products have further been enabled to gather complementary data of high translational relevance [72]. In particular, it was underscored that several formulation options (e.g., based on gels or solid scaffolds)

are adequate for the clinical delivery of FE002 primary chondroprogenitors in a viable and functional form (Table 3).

Table 3. Descriptive listing of the various cell-based product formulation options investigated for primary FE002 chondroprogenitors, as studied under the Swiss progenitor cell transplantation program. The reported diversity in formulation options and technological approaches has confirmed the versatility of the considered biologicals for clinically compatible cytotherapeutics. ACAN, aggrecan; COL, collagen; CRIS, compression released-induced suction; EGDMA, ethylene glycol dimethacrylate; GAG, glycosaminoglycan; GelNB, gelatin norbornene; GLP, good laboratory practices; HA, hyaluronic acid; HEMA, 2-hydroxyethyl methacrylate; Hep, heparin; PEGdiSH, poly(ethylene glycol)dithiol; TG, transglutaminase; TGF-β1, transforming growth factor β1; TRPV4, transient receptor potential channels 4.

Cell Scaffolds/Cytotherapeutic Product Formulation Options	Summary of the Investigated Endpoints (Technical, Functional)/Experimental Data	References
1. HA-TG	Good cellular viability in gels; good chondrogenic potential assessed by *ACAN* and *COL2* gene expression; reported COL2 deposition and increase in compressive modulus. Gel attributes impact cell morphology, proliferation, and chondrogenic potential.	[77,102]
2. Optimaix 3D ± alginate	Homogeneous cellular distribution throughout the scaffold; good in vitro chondrogenic potential assessed by gene expression, GAGs quantification, and immunohistology. Reported absence of hypertrophy markers. Increase in compressive modulus over time. Samples were tested in vivo in mouse subcutaneous implantation.	[76]
3. Novocart Basic	Good cellular distribution throughout the scaffold.	[76]
4. pHEMA scaffold crosslinked EGDMA-fibronectin	Good cellular adhesion and viability. Impact of the gel's level of dissipation on the cellular differentiation potential. Cells resist to seeding protocols using CRIS method (i.e., compression released-induced suction).	[73,74]
5. HA-TG/hep ± TGF-β1	Good cellular viability and proliferation potential maintenance. TGF-β1 concentration and sustained release influence the proliferation and chondrogenic potentials. Compression modulus increases over time in a TGF-β1 dose-dependent manner.	[78]
6. GelNB-PEGdiSH	Good cellular viability maintenance after microencapsulation protocol. Good cell migration potential in the gel. Higher chondrogenic gene induction in microencapsulation versus bulk encapsulation. Higher GAGs deposition in bulk encapsulation, but matrix quality is better with microencapsulation. Samples were tested in vivo in mouse subcutaneous implantation.	[79]
7. Lactoprene combined to HA-TG	Good cellular viability and proliferation potential in the scaffold. Good chondrogenic potential with COL2 deposition. Increase in compressive modulus during cellular differentiation. Samples were tested in vivo in mouse subcutaneous implantation.	[80]
8. pHEMA functionalized with RGD peptides	Cellular adhesion and proliferation potentials are preserved at 32.5 °C and 37.0 °C. Chondrogenic potential is directly influenced by external environmental stimuli (i.e., loading and temperature). Chondrogenic gene expression is increased by loading and self-induced heating (i.e., 32.5 °C–39.0 °C). TRPV4 channel expression is increased by mechanical loading and self-heating. Calcium signalling is involved in chondrogenic genes *ACAN*, *COL2*, and *SOX9* induction.	[75]
9. Chondro-Gide	Specific cellular distribution in the scaffold. Cryopreserved cells may be thawed and seeded on the matrix extemporaneously before implantation. Samples were tested in vivo in a GLP model of goat full thickness chondral defects.	[72,76]

Notably, several in vitro studies have been enabled to gain better comprehension and knowledge about the mechanobiology of the considered FE002 primary progenitor cells and about the influence of formulation and environmental parameters on cellular function/potency (Table 3) [72–79]. Furthermore, several translational studies have been enabled to verify combination product safety and function in vivo in diverse complex settings [72,76,79,80]. Overall, the available reports have underscored the versatility of the

considered FE002 primary chondroprogenitors for cytotherapeutic product formulation, with effective resistance to various physical and chemical stresses in multiple experimental environments (Table 3). Based on existing clinical practices of ACI, two formulation types may tangibly be further considered for the clinical investigational use of allogeneic FE002 primary chondroprogenitors, namely an injectable hydrogel carrying the cellular payload (e.g., cellular suspension, cell spheroids), or a three-dimensional tissue-engineered chondral graft (e.g., cell culture and chondrogenic induction on a bilayer collagen scaffold) [72,79,80]. Therefore, depending on the therapeutic indication or the severity of the orthopedic affection, the diversified cytotherapeutic approach may be modulated regarding product formulation and delivery to fit clinical needs (Figure 4).

7. FE002 Primary Chondroprogenitor Preclinical Safety Evidence: Consistency of Product Innocuity in Various Animal Models

In complement to the in vitro characterization/qualification data available for the considered FE002 primary chondroprogenitors, several in vivo studies were performed and may be aggregated to robustly confirm the safety parameters of the cytotherapeutic materials, in view of their further translational and clinical uses (Table 4).

Table 4. Summarized descriptive listing of the various in vivo models used to study the considered FE002 primary chondroprogenitors and the related cytotherapeutic product prototypes under the Swiss progenitor cell transplantation program. CAM, chorioallantoic membrane model; CRP, C-reactive protein; ECM, extracellular matrix; GLP, good laboratory practices; IL, interleukin; SAA, serum amyloid A protein; SAP, serum amyloid P component; TNF, tumor necrosis factor.

In Vivo Model & Study Type	Summary of the Investigated Endpoints (Safety, Biocompatibility)/Experimental Data	References
1. CAM model	No observed embryotoxicity, no observed angiotoxicity of non-viable cellular materials.	[72]
2. NU/NU nude mice	Cell-seeded scaffolds implanted subcutaneously for 8 weeks. Scaffolds retained ECM. No observed scaffold mineralization or vessel infiltration.	[76]
3. NSG, nude/hu-NSG, C57/BL/6 mice	Cell-seeded scaffolds implanted subcutaneously for 4 weeks. No observed adverse events (e.g., necrosis, oedema, hyperemia). Fibrous capsule formation (i.e., thicker in C57/BL/6 model). No increase in CRP levels. Reduction in SAA and SAP levels compared to empty scaffolds (i.e., except in C57/BL/6 model). Macrophages and T cell recruitment around the scaffolds in C57/BL/6 model, but absent or low in other mouse models. No observed induction of IL-1β, IL-4, IL-6, IL-10, and TNF-α.	[77]
4. NU/NU nude mice	Cell-seeded scaffolds implanted subcutaneously for up to 5 weeks. No observed toxicity. Fibrous capsule formation around the scaffolds. Resistance to vascularization with microencapsulated cells. Regenerated matrix quality documented as being better with microencapsulated cells.	[79]
5. Nude mice	Cell-seeded scaffolds implanted subcutaneously for 6 weeks. Small fibrous capsule formation around the scaffolds. No vascularization within the samples and biodegradation of lactoprene.	[80]
6. Goat model, GLP study	Full thickness chondral defect of the knee. GLP study over 3 months. No test-item related mortality. No observed significant adverse reactions (i.e., local or systemic) in vivo. No changes in monitored clinical signs (i.e., lameness, body weight, neurological). A portion of the human cells were found to have engrafted locally in the host.	[72]

Importantly, the absence of toxicity, immunogenicity, or tumorigenicity of the considered FE002 primary chondroprogenitors was demonstrated within the retained in vivo experimental setups, among which a large animal GLP study (Table 4) [72]. The aggregation of the available and published in vivo safety-related evidence has enabled a positive assessment of the applicability of FE002 primary chondroprogenitors for further translational investigation and pilot human clinical trials [72].

8. Regulatory-Oriented Considerations for Allogeneic Tissue Engineering Products Containing Viable FE002 Primary Chondroprogenitors

Clinical cytotherapeutic applications of bioengineered cartilage grafts containing viable primary chondroprogenitors are regulated by legal dispositions on standardized transplant products (TrSt) in Switzerland [72]. Due to burdensome regulatory constraints (i.e., frequent framework and guideline shifts, high direct costs) for novel product development, limited numbers of sponsors have been investigating and commercializing orthopedic TrSt or cATMP protocols/products for cartilage (e.g., Spherox, CO.DON, Leipzig, Germany) in recent years [64,68]. Notably, examples of authorization expiration for commercialized protocols or products (e.g., MACI in the EU, Genzyme Europe, Amsterdam, The Netherlands) have been documented [103]. Furthermore, some products have been withdrawn by the manufacturer (e.g., ChondroCelect, TiGenix NV, Leuven, Belgium), despite obtained and valid market authorizations [103,104]. These examples tangibly illustrate the difficulty of integration of such innovative cell-based therapeutic approaches in current healthcare systems (i.e., for a variety of reasons, e.g., lack of efficacy, unfavorable cost–benefit ratios, extensive manufacturing delays), despite sizeable clinical need and demand [103]. In addition, several technical and logistical elements (i.e., cryogenic storage and cold chains) and clinical bottlenecks (e.g., low rates of therapeutic cell engraftment) must be considered early on during cytotherapeutic product development phases [72,105].

Notwithstanding, the current state-of-the-art as regards the human cytotherapeutic use of allogeneic FE002 primary chondroprogenitors for cartilage tissue engineering is at the crossroad of large animal models and first-in-man investigational use [72]. Critical aspects of priority interest to regulators and health authorities in view of authorizing a corresponding phase I clinical trial pertain mainly to the quality of manufacturing processes and the demonstration of product safety [70–72]. As concerns the quality of a specified product/protocol, recent harmonization in requirements for GMP manufacturing activities has led to technical updates and the current availability of several contract manufacturers across Europe [99]. With regard to the safety of a novel cytotherapeutic combination product containing allogeneic FE002 primary chondroprogenitors, the use of a registered medical device as a cell scaffold (e.g., Chondro–Gide) may alleviate part of the regulatory scrutiny, as compared to the development of a proprietary matrix/scaffold [72]. As concerns the safety of the cellular active substance itself, the combination of in vivo studies and GMP qualification/release testing results appears significant to form an appropriate preclinical assessment.

Importantly, clinical workflow parameters of allogeneic cytotherapies and cartilage tissue engineering products present significant potential advantages over ACI in its various generations (e.g., reduced operative burdens, no autologous biopsy harvest, no manufacturing-related delays) (Figure 4). Therefore, building on the large available clinical hindsight in ACI (e.g., 5-year follow-up with quantitative endpoints/outcomes), appropriate efficacy targets may be specified for novel allogeneic orthopedic approaches [106–114]. However, as regards the preclinical efficacy evaluation of novel cell or gene-based orthopedic products, high methodological inhomogeneity has been documented and no consensus is currently available [70]. In detail, very few GLP animal studies were reportedly performed (i.e., within market approval procedures) for FDA-authorized cell and gene therapy products [70]. Furthermore, as many patient-reported scored outcomes are used in orthopedics, the need for in vivo preclinical efficacy data has been recently directly challenged, for the rationalization of animal experimentation and resources [70]. Therefore,

based on the growing preclinical data on allogeneic orthopedic cell therapies (e.g., based on FE002 chondroprogenitors) and specific historical cases of documented clinical evidence (e.g., Invossa) of therapeutic success, a transition from autologous to allogeneic cell-based approaches is currently well underway [72].

9. Conclusions and Prospects

Based on the extensive clinical hindsight available for autologous cell therapies in orthopedics and the growing preclinical experience around allogeneic applications, high interest is currently set on the translational development of novel products and protocols for optimized cartilage repair/regeneration. The compilation of the published body of knowledge around FE002 primary chondroprogenitors has confirmed the high versatility of such robust biological materials within translational approaches of chondropathy therapeutic management. Multicentric preclinical research under the Swiss progenitor cell transplantation program has notably demonstrated that such standardized biologicals could be safely and consistently used in various orthopedic product formulations for allogeneic bioengineering approaches. These elements were further confirmed by international literature reports on the similar potential therapeutic uses of chondroprogenitors or derivatives. Clinically, standardized transplants containing FE002 primary chondroprogenitors may potentially be applied in cases of volumetric tissue replacement (e.g., extensive chondral/osteochondral defects of the knee) or localized chondral affections and pathologies (e.g., mild to moderate osteoarthritis). Overall, the use of safe and standardized cell sources, such as FE002 primary chondroprogenitors, and appropriate quality-oriented manufacturing and clinical approaches constitute a tangible working basis for the further translational development of allogeneic orthopedic cytotherapies.

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Abbreviations

ACAN	aggrecan
ACI	autologous chondrocyte implantation
ATMP	advanced therapy medicinal product
CAM	chorioallantoic membrane model
cATMP	combined advanced therapy medicinal product
Col	collagen
CPP	critical process parameter
CRIS	compression released-induced suction
CRP	C reactive protein
DMSO	dimethyl sulfoxide
ECM	extracellular matrix
EGDMA	ethylene glycol dimethacrylate
EOPCB	end of production cell bank
EU	European Union
FBS	fetal bovine serum
FDA	US Food and Drug Administration
GAG	glycosaminoglycan
GelB	gelatin norbornene
GLP	good laboratory practices
GMP	good manufacturing practices
HA	hyaluronic acid
HEMA	2-hydroxyethyl methacrylate
Hep	heparin
KPP	key process parameter
IL	interleukin
IPC	in-process control
MCB	master cell bank
PCB	parental cell bank
PEGdiSH	poly(ethylene glycol)dithiol
Ph. Eur.	European pharmacopoeia
PPC	post-process control
SAA	serum amyloid A protein
SAP	serum amyloid P component
TG	transglutaminase
TGF	transforming growth factor
TNF	tumor necrosis factor
TRPV4	transient receptor potential channels 4
TrSt	standardized transplant product
USA	United States of America
WCB	working cell bank

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