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Opinion

Biologics and cardiac disease: challenges and opportunities

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Biologics are revolutionizing the treatment of chronic diseases, such as cancer and monogenic disorders, by overcoming the limits of classic therapeutic approaches using small molecules. However, the clinical use of biologics is limited for cardiovascular diseases (CVDs) , which are the primary cause of morbidity and mortality worldwide. Here, we review the state-of-the-art use of biologics for cardiac disorders and provide a framework for understanding why they still struggle to enter the field. Some limitations are common and intrinsic to all biological drugs, whereas others depend on the complexity of cardiac disease. In our opinion, delineating these struggles will be valuable in developing and accelerating the approval of a new generation of biologics for CVDs.

Highlights

Cardiovascular diseases represent the leading cause of death globally. However, biological drugs, which stand to be real gamechangers in other medical sectors such as cancer and immune and genetic diseases, struggle to enter the cardiovascular field.

Biological drugs, or biologics, are medicinal products that are produced from living organisms and include recombinant proteins, gene therapy, and cell therapies. Their manufacture is far more complex than small-molecule drugs.

Specific features of cardiac diseases may represent a challenge for the successful application of biologics in clinical settings. Overcoming these challenges may offer unprecedented opportunities to radically change the toolbox of drugs to treat cardiac diseases, with obvious societal and economic implications.

Biological drugs struggle to enter the cardiovascular field

Biological drugs, or biologics, are medical products that are produced from living organisms or contain their components. They mainly include recombinant proteins, gene therapy and cell therapy products. Because their manufacture requires the use of living cells or organisms, their production is much more complex and expensive than that of the majority of chemical drugs and small molecules, which constitute >90% of the current pharmacopeia[i]. Despite this major drawback, the number of biologics approved by the FDA has constantly risen over the past 12 years[ii], with a parallel growth in their market share[iii]. These ascending trends are justified by novel opportunities offered by biologics in multiple medical sectors where small molecules have either been ineffective or displayed severe side effects. The largest impact of biologics has been obtained in oncology, with more than 20 monoclonal antibodies (mAbs) routinely applied in the treatment of several types of cancer[iv]. Moreover, the use of **chimeric antigen receptor (CAR) T cells** (see Glossary) offers – for the first time – a safe alternative to chemotherapy for patients affected by leukemia and lymphomas [1]. Biologics in the form of gene therapy products have provided the first therapeutic option for previously incurable monogenic diseases such as inherited blindness[v] and spinal muscular atrophy[vi]. Finally, cell therapy has progressed remarkably over the past decade in the field of regenerative medicine, particularly for skin and cornea regeneration[vii]. This offers a unique opportunity to treat large burns, in addition to possible combination with gene therapy in the treatment of life-threating hereditary diseases such as epidermolysis bullosa [2].

It is thus surprising that biological drugs, which stand to be game changers in providing therapy for otherwise incurable diseases in other medical sectors, struggle to tackle CVDs, which still represent the leading cause of death globally[viii]. At present, CVDs are largely treated with small molecules that offer palliative support and preserve residual organ function [3], but cannot interfere with the complex mechanisms of disease onset and progression.

Over the past 5 years, multiple studies have assessed the efficacy of biologics in inducing six major biological processes that might transform the current capacity to cure patients affected

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by CVDs. These comprise: (i) inducing cardioprotection; (ii) promoting **angiogenesis**; (iii) inhibiting excessive fibrosis; (iv) improving contractility; (v) reducing major risk factors (i.e., atherosclerosis); and, in the best possible scenario, (vi) stimulating cardiac regeneration (Figure 1). While encouraging results have been obtained in small-animal models of CVDs [4], most of these approaches have essentially failed once they have reached either the large-animal or clinical stage [4].

We believe that delineating the reasons for these failures will be valuable in developing solutions to overcome the existing limitations and thus to increase the chance of success of future research and clinical studies assessing the safety and efficacy of novel biological drugs for cardiac disease.

Biologics for heart disease: the state of the art

Here, we describe the few biologics that have been developed in the cardiovascular field over the past 4 years, which have either entered the clinical arena or have been tested in large animals and are therefore expected to soon reach human application. These include recombinant proteins and mAbs, and gene and cell therapy products as detailed in the following subsections.

Recombinant growth factors and mAbs used to treat cardiac ischemia and its associated risk factors

Recombinant proteins have been the first type of biological drugs considered for use in the therapy of patients affected by heart disease.

Starting from the pioneering studies led by Jeffrey Isner aimed at infusing angiogenic proteins to induce angiogenesis as a treatment for cardiac ischemia, many trials have delivered recombinant growth factors to the heart, with the aim of either exerting cardioprotection or inducing bone marrow (BM) cell mobilization [5]. Most of these trials did not achieve successful results, as it soon became evident that the half-life of recombinant proteins *in vivo* is too short to result in any persistent therapeutic effect [6]. Thus, it is unsurprising that only a few additional trials have started in recent years. Among these, the Randomised Trial Evaluating the Safety and Efficacy of a Single Low Dose of Intracoronary Insulin-like Growth Factor-1 Following Percutaneous Coronary Intervention for ST-Elevation Acute Myocardial Infarction (RESUS-AMI) Phase 1 study entailed the administration of insulin-like growth factor 1 (IGF1) in 47 patients with **ST elevation myocardial infarction (STEMI)** after percutaneous coronary intervention, without achieving significant improvement in cardiac function [7]. A new Phase 3 trial to assess the efficacy of systemic infusion of neuregulin (NRG) in chronic **heart failure (HF)** patients currently aims to enroll 1600 patients, despite the negative results obtained by similar approaches over the past 15 years (NCT03388593).

More promisingly, the mobilization of hematopoietic cells using recombinant proteins such as granulocyte colony-stimulating factor (G-CSF) is expected to work systemically. Two recent publications support the efficacy of the early administration of G-CSF after **acute myocardial infarction (AMI)** in improving cardiac function for up to 10 years [8,9]. How these positive data compare with the discouraging results of intracardiac delivery of BM cell therapy in the heart remains an open question and suggests that complex cardioprotective mechanisms, on top of a direct contribution to cardiac repair, are relevant.

mAbs constitute a special class of recombinant proteins. Due to their safety, specificity, and efficient manufacture, mAbs stand to be game changers for multiple disorders, including cancer and infectious and autoimmune diseases [10]. However, their application in the cardiovascular field remains in its infancy and is largely directed at blunt excessive inflammation in patients affected by AMI and HF. In the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) trial, a mAb specific for IL-1β reduced **major adverse cardiovascular events (MACEs)** in

Glossary

3D bioprinting: use of 3D printing technologies to combine cells, growth factors, and biomaterials to fabricate biomedical parts with the aim of reproducing the features of natural tissues.
Acute myocardial infarction (AMI): myocardial necrosis resulting from the acute obstruction of a coronary artery.
Allogenic cells: cells harvested from an individual different from the patient.
Angiogenesis: the process through which new blood vessels form from pre-existing vessels.
Autologous cells: cells harvested from the same individual.
Chimeric antigen receptor (CAR) T cells: T cells that have been genetically engineered to produce an artificial T cell receptor combining both antigen-binding and T cell-activating functions in a single receptor; CAR T cells are expected to effectively target and destroy all cells expressing the specific antigen.
Ejection fraction (EF): the volumetric fraction of blood ejected by each ventricle with each cardiac contraction.
Heart failure (HF): clinical syndrome characterized by dyspnea or exertional limitation due to failure of the heart to pump enough blood to meet the body's needs. In HFrEF, the heart muscle is not able to contract adequately and therefore expels insufficient oxygen-rich blood into the body. However, it is possible to observe HF symptoms in patients with preserved EF [HF with preserved EF (HFpEF)], also known as diastolic HF. In this case, the heart muscle contracts normally; however, its thickening reduces the blood volume inside the ventricle.
Hydrogels: 3D networks of polymers with hydrophilic properties that keep an organized structure when immersed in a liquid; they can comprise either synthetic polymers or natural proteins, such as those of the extracellular matrix.
Induced pluripotent stem cells (iPSCs): pluripotent stem cells generated directly from somatic cells through the introduction of four genes encoding transcription factors able to reprogram them back into an embryonic-like pluripotent state.
Low-density lipoprotein cholesterol (LDL-C): estimated measure of how much cholesterol is transported by all LDL particles; as LDL particles deliver fat molecules to cells, they are involved in

patients with a history of AMI [11]. Additional Phase 3 studies using mAbs targeting IL-6 (tocilizumab), CD20 (rituximab), and phosphorylcholine (ATH3G10) are currently ongoing (NCT03004703; NCT03332888; NCT03991143) [12].

In addition to these strategies directed at the heart itself, a few approaches try to address major risk factors for cardiac diseases, such as hypercholesterolemia. Among these are the delivery of recombinant apolipoprotein A1 [13] and the use of a mAb against proprotein convertase subtilisin/kexin type 9 (PCSK9), which effectively reduced MACEs in high-risk patients [14]. The EVOlocumab in Stable Heart Failure with Reduced Ejection Fraction of Ischemic Etiology (EVO-HF) clinical trial (NCT03791593) will specifically assess whether patients with HF with reduced **ejection fraction (EF) (**HFrEF) also benefit from PCSK9 inhibition.

Gene therapy products for cardiac disease include protein-coding genes and noncoding RNAs

While gene therapy is becoming the treatment of choice for several genetic conditions, two gene therapy products have reached clinical approval and the market phase in the CVD sector. Glybera® was the world's first gene therapy product, approved in 2012 for patients affected by lipoprotein lipase deficiency, which leads to hypercholesterolemia and thus increases the risk of MACEs [15]. This, however, was one of the most expensive medicines in history (US$1 million per dose) and was withdrawn in 2017 for lack of demand [16]. The other product is Collategene®, a DNA plasmid encoding hepatocyte growth factor (HGF), delivered not to the heart but to patients affected with critical limb ischemia with the aim of inducing angiogenesis and thus favoring ulcer healing. Collategene® has received conditional and time-limited approval restricted to Japan [17]. Two new clinical trials are scheduled to start in the coming years aimed at evaluating the efficacy of an adenoviral vector expressing HGF in patients with AMI (NCT02844283; NCT03404024).

Despite these two relative successes, an enormous effort has been made over the past 20 years in the development of gene therapy approaches targeting the myocardium, with the aim of either inducing new blood vessel formation in the ischemic heart or improving cardiac contractility. Both strategies have reached the clinical phase, with over 150 trials of therapeutic angiogenesis, all unsuccessful, and tens of studies aiming to improve cardiac contractility in HF patients. In this latter case, the main strategy for intervention was the delivery of an adeno-associated vector (AAV) for the expression of the calcium pump sarco/endoplasmic reticulum Ca^{2+}-ATPase (*SERCA*)*2A*, which is known to be consistently downregulated in failing hearts [18]. However, none of these studies has so far provided positive and conclusive results. As the failure of AAV-*SERCA2A* gene therapy could be reasonably attributed to either the inclusion of patients with multiple forms of HF or the presence of pre-existing anti-AAV antibodies, two new trials are ongoing to assess the efficacy of AAV-*SERCA2A* specifically in patients affected by HfrEF and the use of a heart-specific chimeric AAV mutant, which is supposed to be more resistant to neutralizing antibodies.

Over the past 10 years, applications of gene therapy in cardiac diseases have extended beyond the transfer of cDNAs to embrace RNA-based therapies, including the delivery of long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), miRNAs, short hairpin RNAs (shRNAs), small interfering RNAs (siRNAs), and locked nucleic acids (LNAs) [19]. The success story of the long-acting first-in-class siRNA-based drug inclisiran targeting PCSK9, a protein involved in the regulation of **low-density lipoprotein cholesterol (LDL-C)** metabolism, represents a blockbuster in the field. The drug is entering the European market for adults with primary hypercholesterolemia [20] based on data from Phase 3 ORION studies [21]. Inclisiran can reduce LDL-C levels by approximately 50% compared with placebo and is expected to impact, in particular, the coronary atherosclerotic burden in these patients. Regarding direct heart delivery, the only clinical trial so far conducted in this field

the formation of atherosclerotic plaques. The common clinical interpretation of blood lipid levels is that high LDL-C is associated with increased cardiovascular risk.

Major adverse cardiovascular events (MACEs): a composite endpoint frequently used in cardiovascular research. While the MACE definition is not always consistent between different clinical trials, it usually refers to the composite of total death, myocardial infarction, stroke, hospitalization because of HF, and need for revascularization, including percutaneous coronary intervention and coronary artery bypass graft.

New York Heart Association (NYHA) functional classification: classification of HF severity based on the intensity of symptoms during physical activity (shortness of breath and/or angina).

ST elevation myocardial infarction (STEMI): myocardial ischemia accompanied by persistent elevation of the ST segment on the electrocardiogram; it usually occurs due to occlusion of one or more coronary arteries, causing transmural myocardial ischemia, which in turn results in myocardial injury or necrosis.

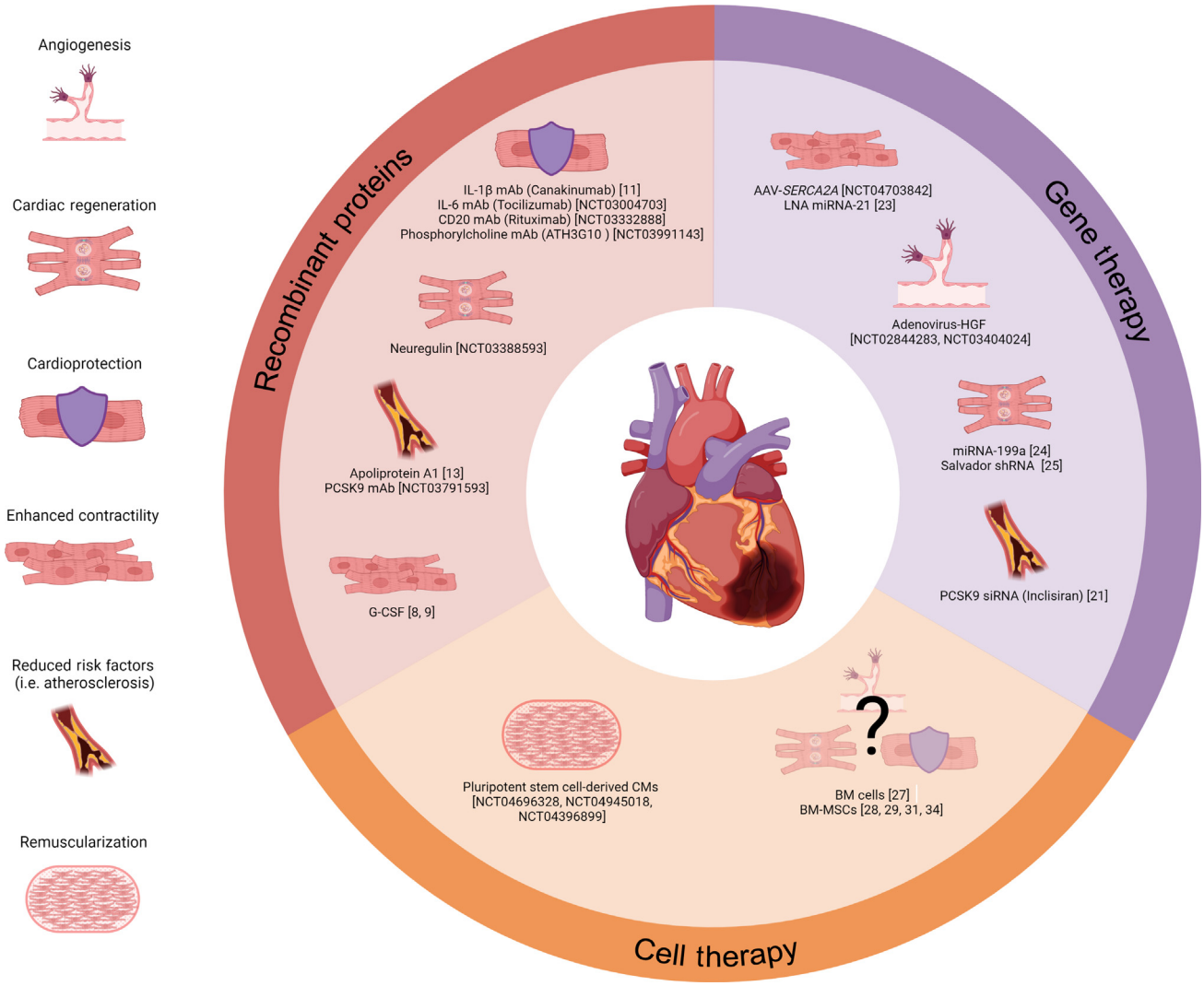

Figure 1. Biologics for cardiac diseases. Schematic representation of the three categories of biologics considered for the therapy of cardiac disorders, with an indication of the major biological processes targeted by each approach and references to the corresponding publications and numbers of clinical trials. In the case of bone marrow (BM) cells and BM mesenchymal stromal cells (BM-MSCs), the question mark indicates a hypothetical mechanism of action. Created with BioRender.com. Abbreviations: AAV, adeno-associated vector; CM, cardiomyocyte; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; LNA, locked nucleic acid; mAb, monoclonal antibody; PCSK9, proprotein convertase subtilisin/kexin type 9; *SERCA2A*, sarco/endoplasmic reticulum Ca^{2+}-ATPase 2A; shRNA, short hairpin RNA; siRNA, small interfering RNA. References used are [8,9,11,13,21,23–25,27,29,31,34].

aimed to antagonize miR-132 in HF patients [22]. While the trial confirmed the feasibility and safety of the approach, evidence of efficacy is still missing. Additional approaches using RNA therapeutics have shown efficacy in large animals, mainly in pig models of ischemia reperfusion injury, which well mimic the human condition; for example, an anti-miRNA-21 LNA suppressed cardiac hypertrophy and improved cardiac function [23] at 1 month after AMI. Even more exciting results were obtained using RNA drugs with the potential to promote cardiomyocyte proliferation and thus cardiac regeneration. These include the AAV-mediated delivery of either human miRNA-199a or a shRNA specific for the Hippo pathway *Salvador* (*Sav*) gene, which resulted in both massive cardiomyocyte proliferation and the generation of new myocardium in the ischemic pig heart [24,25].

Attempts in cell-based therapies for cardioprotection and remuscularization

More than 200 clinical trials of cell therapy for cardiac diseases, in particular for AMI, and nearly 50 meta-analyses have been performed over the past 20 years [26]. Most of these have assessed the efficacy of mesenchymal stromal cells (MSCs) despite unclear evidence of their mechanism of action, which could include anti-inflammatory, antiapoptotic, antifibrotic, and proangiogenic activities. Only a few of these trials reached the end of the study and gave conclusive results, mainly because of the small number of participants. The last metanalysis considered 38 randomized controlled trials involving 1907 participants who received BM-derived stem/progenitor cell therapy for cardiac repair and concluded that these cells do not provide any significant benefit to patients [27].

Against this background, two large randomized, double-blind, placebo-controlled trials were performed over the past 4 years and entailed the intracardiac delivery of BM-derived MSCs in patients with advanced, ischemic HF [**New York Heart Association (NYHA) functional classification** II/III]. The MSC-HF trial recruited 55 patients and showed an increase in left ventricle EF by 6% in the MSC group compared with placebo but no changes in NYHA class [28]. More recently, the Combination of Mesenchymal and c-kit$^+$ Cardiac Stem Cells as Regenerative Therapy for Heart Failure (CONCERT-HF) study enrolled 125 patients and showed improved quality of life and a decline in hospitalization for HF in those who received MSCs either alone or in combination with c-kit$^+$ cardiac cells, but no changes in either left ventricle EF or scar size [29].

These results led to additional skepticism on the real therapeutic relevance of this approach for patients affected by ischemic HF, as one would expect that an effective treatment invariably results in a clear improvement in both cardiac function and symptoms in multiple patient populations. These inconsistencies could be due, at least in part, to the use of autologous versus **allogenic cells** in the MSC-HF and CONCERT trials, respectively. While **autologous cells** have a safer profile in terms of engraftment and survival, the use of allogenic cells, which are potentially younger, could lead to better and more reproducible results [30].

An interesting suitable target for cardiac cell therapy is represented by the niche of patients with severe ischemic heart disease experiencing refractory angina. Available evidence suggests functional and clinical benefits of BM-derived mononuclear or CD34$^+$ cells when they are delivered intramyocardially into the ischemic territories of the left ventricle [31].

Beside the use of BM-MSCs, in the past few years encouraging results were obtained with pluripotent stem cells, both embryonic stem cells (ESCs) and **induced pluripotent stem cells (iPSCs)** to treat severe HF [32,33]. Transplantation of Human Embryonic Stem Cell-derived Progenitors in Severe Heart Failure (ESCORT) was the first clinical trial to assess the safety of the transplantation of cardiac-committed progenitor cells derived from human ESCs [34]. At 1-year follow-up, the procedure was found to be safe, without any arrhythmias or evidence of tumor formation. More recently, three studies are using iPSC-derived cardiomyocytes to promote remuscularization and enhancement of myocardial performance in the failing heart (NCT04696328; NCT04945018; NCT04396899).

Why the struggle?

As highlighted in the preceding text, the impact of biologics in the therapy of heart disease remains limited (Figure 2). This raises two important questions. First, why is this so, despite the high incidence and mortality of these diseases and thus the huge clinical and societal demand for innovative therapies? Second, what are the opportunities that may derive from understanding the struggle faced by past and current approaches?

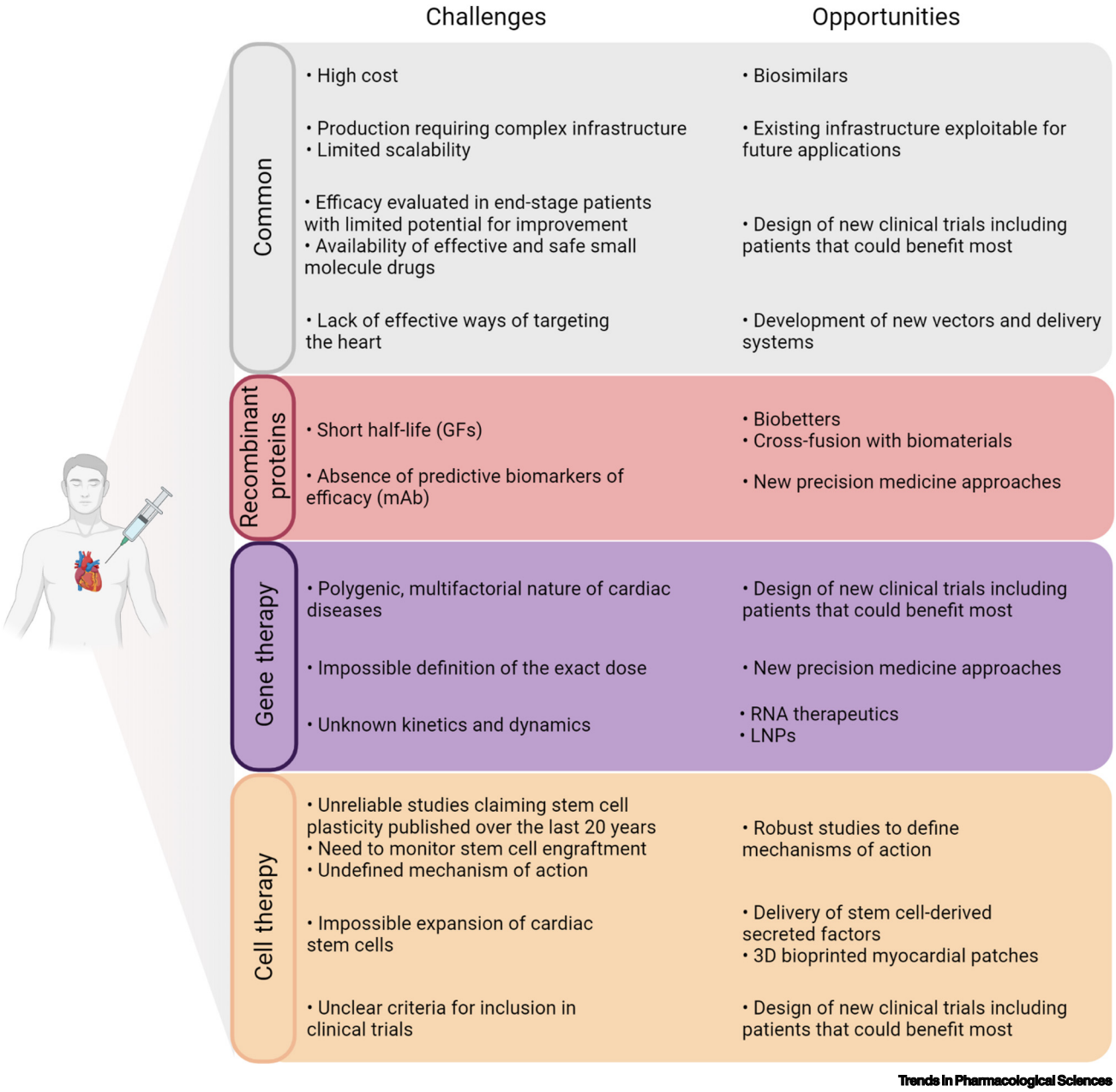

Figure 2. Major challenges faced by biologics for cardiac diseases and opportunities to overcome them. While some challenges appear to be common and equally important for all biologics, others are particularly relevant for one specific category. In the case of recombinant proteins, challenges that are more relevant for monoclonal antibodies (mAbs) and growth factors (GFs) are indicated. Opportunities that may help in overcoming each challenge are indicated in the same line. Created with BioRender.com. Abbreviation: LNP, lipid nanoparticle.

A partial explanation could reside in the intrinsic limits of biologics, such as the high costs and complex infrastructure and manufacture required for their production, which often limit their scalability for the treatment of large numbers of patients.

However, mAbs are widely applied to myriad cancer patients [35], and during the COVID pandemic we have witnessed the accelerated development and market uptake of numerous

biologics (i.e., both adenoviral vectors and mRNA-based vaccines, SARS-CoV2-specific mAbs) [36,37]. A recent analysis came to the counterintuitive conclusion that the median, total, premarket development time does not differ between biologic and small-molecule drugs and appears to be even shorter for biologic drugs using the Merck Index (10.6 vs 12.6 years) [38].

Thus, some peculiar features of cardiac diseases probably limit the application of biologics specifically for the heart.

First, cardiac diseases are currently treated with safe and cost-effective drugs and thus the move to biological drugs could be less willingly accepted compared with cancer, for which chemotherapeutics are usually more expensive and fraught with major side effects [39]. Similar considerations could be applied to immune diseases, for which we do not have many therapies other than steroids, again causing several side effects [40].

In addition, cardiac diseases are rather complex, depending on the coordinated action of multiple factors that cooperate in space and time in both disease onset and progression [41]. Thus, the definition of the most relevant targets is a major challenge. Despite numerous attempts to use gene therapy to interfere with the pathogenesis of complex disorders, not only in the cardiovascular field but also in neurodegeneration, its success remains limited to genetic conditions such as severe immunodeficiencies, hemophilia, and hereditary blindness, in which the therapeutic gene is well defined and the only one required to cure the disease [42]. Similarly, cell therapy works well when stem cells are implanted in the same organ from which they are derived (i.e., hematopoietic stem cell transplantation to treat immune diseases, epithelial stem cells to regenerate the skin or the cornea [43,44]). Unfortunately, the heart lacks a population of stem cells that can be expanded *ex vivo* and implanted back into the patient [45]. The possibility of injecting stem cells to regenerate other tissues different from their own origin initially generated enthusiasm in the scientific community, but the real stem cell plasticity has been severely challenged over the past few years [45]. In the specific case of the heart, extreme confusion has been created in the field by a plethora of unreliable studies, which claimed the potential of either BM cells or circulating progenitor cells to give rise to new blood vessels and new myocardium in response to local stimuli. While these concepts have been clearly questioned in more recent years, initial studies have led to several unsuccessful clinical trials, with an impressive loss of money and credibility [45]. Other fields, in which the translation of biologics from small-animal models to humans has progressed more slowly but rigorously, are now more advanced.

Another element that may have prevented the progression of biological drugs for cardiac therapy is the selection of patients to be included in early clinical trials. Since multiple therapeutic opportunities exist for most cardiac diseases, new biologics are tested on end-stage patients, in whom it may be difficult to establish efficacy. For example, most of the studies in which a biologic provided benefit in cardiac function showed a modest improvement in EF, in the 3–7% range [4,7,22,28]. This could result in differing clinical outcomes depending on the severity of the underlying disease; hence, the relevance of identifying the group of patients that can benefit most from the treatment. When designing new trials – for example, those entailing the use of iPSC-derived cells – it will be of paramount importance not to repeat the same mistakes committed with BM-derived cells [45], but to clearly define criteria for patient inclusion, ensure appropriate power to reach significant results, consider the long-term outcome, monitor cell survival after implantation, and, most importantly, progress in parallel with preclinical research and define the mechanism of action. Ideally, patient selection could be driven by an individualized approach, similar to precision medicine for small molecules. The possibility of identifying the patient population that has the greatest chance of responding to a specific biologic would require the definition of

predictive biomarkers. For example, mAbs targeting human epidermal growth factor receptor-2 (HER2) are selectively administered in cancers overexpressing this receptor [46]. Similar approaches could be proposed to identify the patients that express the highest levels of PCSK9 or other targets for which mAbs are available.

Another major issue for most biologics is their short half-life, particularly when delivered systemically, and the lack of effective ways of targeting the heart. This is particularly relevant for recombinant proteins and cells, but also for genes delivered as either nude nucleic acids or adenoviral vectors. Recent approaches using biomaterials for local, slow, and controlled release could offer promising opportunities in the near future [47]. **Hydrogels**, in the form of either injectable solutions or patches, represent the most promising platform for rapid and effective clinical translation, as in the Biological Ventricular Assist Tissue (BioVAT)-HF clinical study, in which iPSC-derived cardiomyocytes are embedded in a collagen hydrogel scaffold prior to application to the cardiac surface (NCT04396899). Both recombinant proteins and small RNAs could be efficiently embedded in hydrogel scaffolds for their gradual, spatially defined, and long-lasting release at the site of cardiac damage. However, the need for an invasive route of administration for these composite materials could limit their clinical application.

For many biologics, the real half-life – but also the dose, kinetics, and dynamics – is not yet precisely known. Different from small molecules that can be easily administered at a precise dose and their concentration measured in multiple biological fluids and tissues over time, to define a precise dosage, biologics for heart therapy (i.e., AAV-*SERCA2A* and most cell therapies) have been administered to patients in a naïve approach without careful checking of the active concentration of the drug in the cardiac tissue and adjustment of its administration in relation to its half-life. Excessive and uncontrolled expression of the transgene could itself be deleterious. For example, AAV-mediated delivery of miR-199a in pig hearts resulted in persistent de-differentiation and proliferation of cardiomyocytes, which formed tumor-like masses leading to fatal arrhythmias [24]. Novel formulations allowing the delivery of synthetic miRNA mimics, for example encapsulated in lipid nanoparticles (LNPs), could represent a valuable option devoid of side effects due to long-term expression associated with AAV vectors [48]. At the same time, cardiac regeneration in the ischemic heart would require adequate revascularization to provide trophic support to the regenerating myocardium. Thus, the combination of proangiogenic and proregenerative strategies is highly warranted in the future.

As an additional note, most of the biologics that have so far entered the clinical arena have been aimed to improve cardiac contractility, promote cardiomyocyte survival despite the harsh ischemia, or regenerate the heart. No approaches have progressed with the aim of interfering with multiple pathogenetic mechanisms that are common to heart diseases. A preclinical study has recently shown the efficacy of a new mAb targeting bone morphogenetic protein (BMP) 1.3 in both promoting the survival of cardiomyocytes and reducing scar stiffness after AMI in a mouse model [49]. While still at an early preclinical stage, this study is relevant because it shows the potential of strategies exerting pleiotropic functions and that are therefore able to tackle multiple aspects of the complex pathogenesis of cardiac diseases. At the same time, it paves the way for a new class of biologics that do not interfere with scar formation and thus avoid the associated risk of cardiac rupture, but rather improve scar elasticity [50].

Future perspectives

In this Opinion, we have highlighted multiple features of biologics that may have so far prevented their successful introduction into the cardiovascular arena (see Outstanding questions).

A glimmer of hope stems from recent progress in the clinical development of biologics compared with small molecules. While both the success rate and the time to regulatory approval were very

unpredictable 10 years ago, recent analysis indicates that biologics currently perform even better than small molecules in some applications [38]. The challenge now is to bring this trend into the cardiac field. How can some of the current limitations be overcome?

For recombinant proteins, the development of biobetters and biosimilars (Box 1) will surely offer novel opportunities for improved performance and cost reduction [51–53]. In addition, the identification of biomarkers able to predict which patients have the highest chance of responding, thus integrating biologics in the context of personalized therapy, could significantly improve their clinical performance. Finally, the cross-fusion of recombinant proteins and biomaterials could significantly prolong their half-life and achieve spatial control of their release, which have so far limited their clinical efficacy.

In the case of gene therapy, major struggles are the efficiency of gene delivery and the definition of appropriate dose. Both limitations could be overcome by the incorporation of the corresponding mRNAs into LNPs, as recently demonstrated for the *in vivo* generation of CAR T cells specifically

Box 1. Biosimilars and biobetters

Off-patent biologics have the promise to fill a potentially large market in follow-on biologics: biosimilars and biobetters [56].

Biosimilars

Once the patent for a biologic expires, it becomes possible to develop and commercialize biosimilars. These are biologics that are similar to a reference molecule in structure, efficacy, safety, target, formulation, dosage, and administration [57].

This means that they are not exact copies of the reference active ingredient, as with small-molecule drug generics. More specifically, the European Medicines Agency (EMA) defines a biosimilar as 'a medicine highly similar to another biological medicine already marketed in the EU (so-called 'reference medicine')'[ix] and the FDA as 'a biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product'[x].

Because of the nature of biological products, as well as the natural variations in their manufacture, biosimilars are regulated differently from generic drugs. This means there might be differences between the biosimilar and the reference product in clinically inactive components. However, a biosimilar must have the same dosage form, route of administration, and strength as the reference product [58]. Similarity must be proven by preclinical and clinical studies demonstrating purity, safety, and efficacy in all of the conditions for which the reference product is licensed [57,58].

As biosimilars usually allow reduction of the cost of treatment by up to 25%, they are expected to become market disruptors for affordability and wider availability. The EU approved the first biosimilar product in 2006. Since then, it has approved 55 biosimilars[xi].

These products constitute about 90% of the injectionables market in Europe. In comparison, the first biosimilar was approved in the USA in 2015, and the drugs currently represent no more than 20% of the market[xii].

Biobetters

These are biologics that perform better than the reference molecule in one or more parameters, while sharing the same target [52]. They are usually designed from existing biological drugs to obtain higher selectivity, stability, and half-life, or lower toxicity and immunogenicity. Among the strategies most commonly used for improvement are changes of the amino acid sequence, glycosylation, bioconjugation with polymers (i.e., PEGylation), humanization, and protein fusion [52].

Biobetters usually require high resources during their research and development pipeline. However, as they have a proven target at hand and the good efficacy of the reference biologics, there is less likelihood of these molecules failing to reach the market [59]. Biobetters are classified as investigational new drugs; thus, they do not have to wait for patents and market exclusivity to expire, and hence provide returns to the manufacturers earlier than biosimilars. Having close similarity with the reference biologic, they can be rarely patented, but they can gain market exclusivity. Because biobetters are by definition more efficient than their reference molecule and biosimilars, they can demand a price premium, in contrast to biosimilars that have to be price sensitive as they are not offering anything new or better to patients [59]. This premium price can be cancelled by the reduced dosing, better half-life of the drug, and longer shelf life. Thus, biobetters are likely to decrease the overall cost of treatment [56].

targeting and eliminating profibrotic cells in a mouse model of AMI [54]. To what extent this approach could be used to develop an mRNA-based vaccine for cardiac fibrosis, similar to that being done for infectious diseases and cancer, remains an intriguing question.

As mentioned previously, BM-MSCs are expected to exert pleiotropic activity, ranging from immune modulation to cardioprotection and angiogenesis. Thus, further studies are warranted to define the relative importance of these mechanisms and the molecules involved in these paracrine activities. These molecules could be delivered directly as a cocktail of therapeutic factors at a precise dose, thus avoiding the use of cells and associated costs and the requirement for cell factories. The challenges associated with cell therapy for remuscularization are even higher, possibly due to the complex architecture of the myocardial tissue and the need for electromechanical coupling. Successful cell therapy approaches so far are essentially limited to blood and epithelial cell transplantation, in which the target tissue has a liquid structure (blood) [43] or comprises a 2D layer (skin and cornea) [44], which is relatively easy to build under standard cell culture conditions. The availability of **3D bioprinting** islikely to offer the opportunity to create personalized myocardial patches to be engrafted into the myocardium [55]. While current approaches merely lay some contractile tissue on the epicardial surface of the infarcted heart, bioprinted 3D cardiac tissues could offer for the first time the opportunity to replace the fibrotic scar with new viable myocardium.

Concluding remarks

We have discussed the major obstacles that might have so far prevented the approval of biologics for cardiac diseases. The scene is surely set to change in the future. What might be the first biologic to enter the clinical stage? In our opinion, mAbs are the prime candidates because of their long, safe, and successful application in many clinical fields and the possibility of administration multiple times at a precise dose. Gene therapy could follow, in the form of RNA-based therapy, which is the only way to deliver nucleic acids at a controlled dose. This is experiencing remarkable momentum, particularly following the COVID19 pandemic. Finally, cell therapy, while possessing the unique potential to rebuild a diseased heart, seems to be the most difficult strategy to offer patients in a safe, reproducible, and scalable manner. Certainly, whatever the regulatory agencies decide in the near future that either confirms or disproves our predictions, the approval of the first biologic for cardiac diseases will surely represent a steppingstone toward the progressive entrance of these classes of therapies in the field.

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Declaration of interests

No interests are declared.

Resources

[i] www.immpressmagazine.com/small-vs-big-understanding-the-differences-between-small-molecule-drugs-and-biologic-drugs/

[ii] www.biopharmatrend.com/post/67-will-small-molecules-sustain-pharmaceutical-race-with-biologics/

[iii] www.forbes.com/sites/theapothecary/2019/03/08/biologic-medicines-the-biggest-driver-of-rising-drug-prices/#1ea11ad918b0

[iv] www.accessdata.fda.gov/scripts/cder/daf/

[v] www.fda.gov/news-events/press-announcements/fda-approves-novel-gene-therapy-treat-patients-rare-form-inherited-vision-loss

[vi] www.fda.gov/news-events/press-announcements/fda-approves-innovative-gene-therapy-treat-pediatric-patients-spinal-muscular-atrophy-rare-disease

Outstanding questions

When will biologics successfully enter the clinical arena in the cardiovascular field?

Can strategies promoting cardiac regeneration be safely applied without the risk of inducing uncontrolled cell proliferation?

What are the mechanisms behind the small but consistent cardioprotective effect of BM cells?

What is the target population of patients with cardiovascular diseases that can benefit most from biologics?

What are the most appropriate outcomes to be used as primary and secondary objectives to document improved cardiovascular function in future clinical studies?

What parameters can be used for cell therapy instead of pharmacokinetic and pharmacodynamic studies traditionally performed for small molecules?

What is the best way of defining the appropriate dose of a biological drug? Is it the dose that is delivered or it should be adjusted based on real-time monitoring of a marker of efficacy (i.e., level of gene expression for gene therapy, downregulation of specific targets for RNA-based therapy, etc.)?

Will future biologics justify their cost?

Will biobetters and biosimilars improve the cost-effectiveness of biologics and render their market more sustainable?

[vii]www.holostem.com/?lang=en

[viii]www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

[ix]www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/multidisciplinary/multidisciplinary-biosimilar

[x]www.fda.gov/drugs/therapeutic-biologics-applications-bla/biosimilars

[xi]www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/multidisciplinary/multidisciplinary-biosimilar

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