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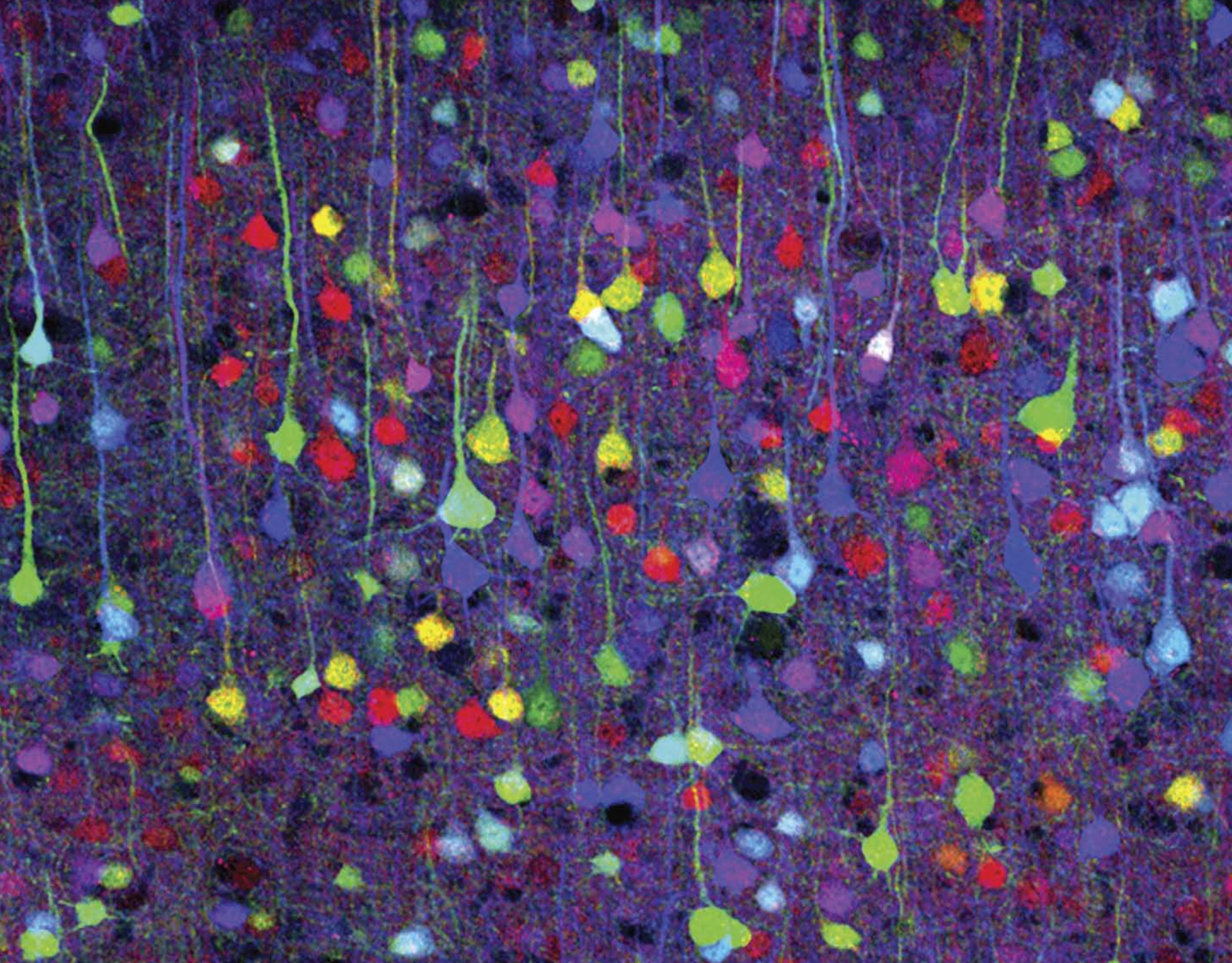

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Developing Lifeline™, a Small-Caliber Autologous Tissue-Engineered Blood Vessel, for Clinical Trials in Germany

By CHRISTIAN VAN DEN BOS

A considerable clinical need exists for alternatives to the autologous vein and artery tissues used for vascular reconstructive surgeries such as coronary artery bypass graft (CABG), lower limb bypass, arteriovenous shunts, and repair of congenital defects to coronary circulation.

So far, devices made from synthetic materials have not matched the efficacy of native grafts, particularly in small diameter applications. Recent advances in cell biology and tissue engineering have introduced the possibility of a living, biological graft that might mimic the functional properties of native vessels.

In this article, we discuss challenges encountered in developing this unique cell-based product as an investigative medicinal product for clinical trials in Germany. In particular, we address issues specific to the manufacturer's process, its facilities, and regulatory requirements for this product. We further discuss the requirements specific to importation of a cell-based therapeutic into the EU. To adequately cope with these issues, we have bundled these services in a new organization, Mares Ltd., located in Germany. It serves to facilitate the legal representation, import and release of this unique cell-based product in accordance with EU regulations.

Introduction

Despite more than 50 years of research examining synthetic materials, native vein and artery segments remain the gold standard for revascularization procedures. Unfortunately, suitable veins and arteries are not always available for peripheral or coronary revascularization and hence, a need exists for small-caliber grafts.

One solution to the problem may be a fully autologous, endothelialized, tissue-engineered blood vessel (TEBV). Conceptually, such a graft would address the main failure modes of synthetics—thrombosis, chronic inflammation (and subsequent restenosis), and infection. Tissue-engineered grafts would also eliminate the co-morbidities associated with vein harvest.

In this report, we review the development of the Lifeline™ vascular graft, a small-caliber TEBV, as investigational medicinal product (IMP), and discuss several of the key issues that are likely to impact cellular products in general.

An Autologous Tissue-Engineered Blood Vessel

Lifeline is based on an approach termed "sheet-based tissue engineering" developed by L'Heureux and coworkers.[1] It comprises sheets of patient's fibroblast and extracellular matrix generated by these and the subsequent use of such sheets to form three-dimensional tissues such as TEBVs.

TEBVs are seeded with the patient's endothelial cells on the luminal surface to provide anti-thrombogenicity found in a native vessel.[2] It is important to note that the Lifeline graft is devoid of synthetic materials, which not only reduces inflammatory responses but also has important regulatory implications. In Germany, autologous products derived from human materials, prior to the application of the advanced therapy medicinal product regulation (ATMP), do not require a marketing authorization once they are legally on the market. And the latter is constituted by, for example, an import permit according to §72a AMG (German drug law), as shown in Figure 1.

The starting material, the patient's own cells in this case, is obtained by harvesting a skin and superficial vein biopsy. Both tissues are extracted and fibroblasts and endothelial cells expanded in separate cultures. Fibroblasts are cultured under conditions conducive to extracellular matrix formation. The resulting cell and extracellular matrix sheets are rolled around a mandrel and matured in culture to promote fusion between layers. The resulting tubular membrane is devitalized by drying and the process of rolling is repeated with a second sheet so as to provide an outer living ply synonymous to the native vessel's adventitia. Following further maturation, the mandrel is removed and the luminal surface of the two-ply TEBV seeded with endothelial cells. The construct is preconditioned prior to implantation for up to one week with flow and pressure. During manufacture, key biomechanical properties are determined and

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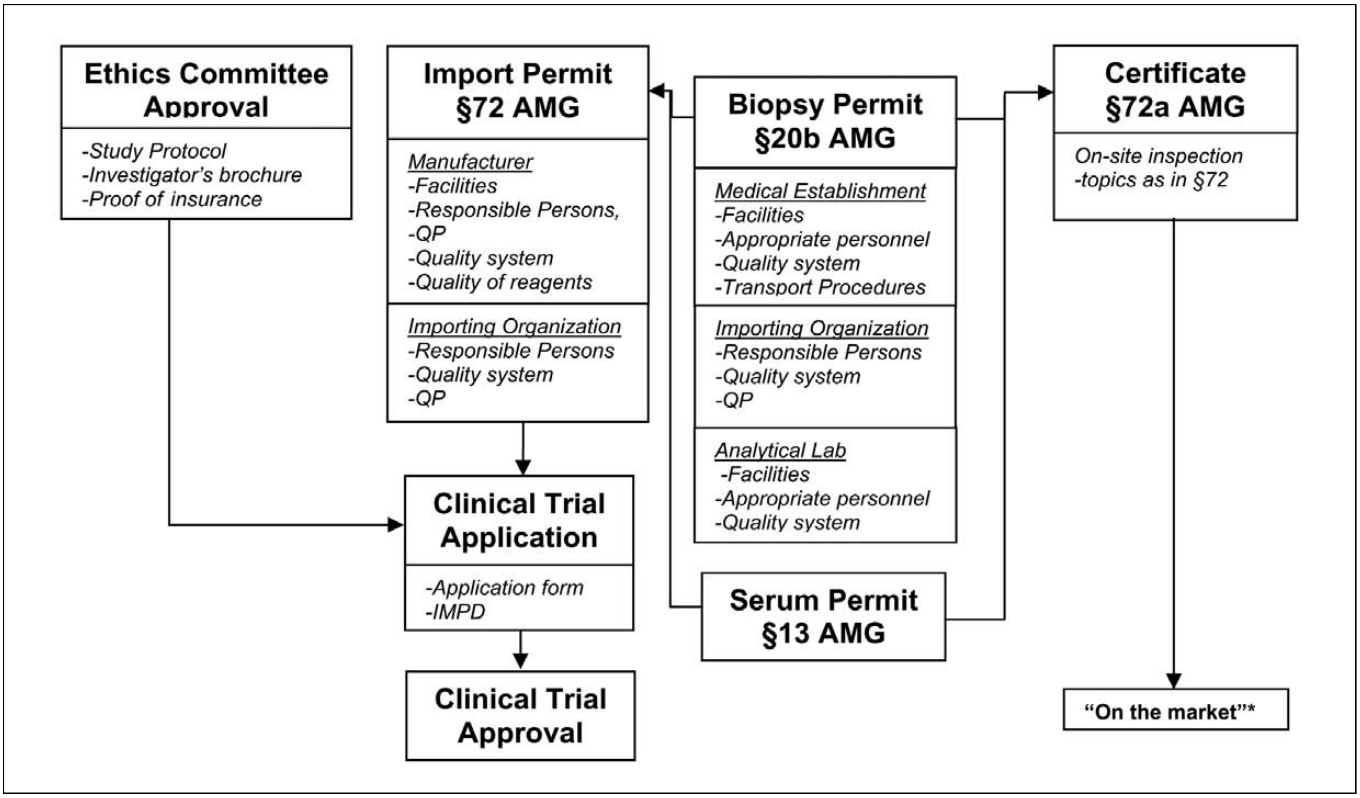

FIGURE 1. Regulatory pathway for approval of a tissue-engineered product in Germany. This schematic primarily describes the pathway to clinical trials. *"On the market" denotes the legal situation through December 2008, which was prior to the application of the Advanced Therapy Medicinal Product (ATMP). Products with a certificate according to §72a AMG may be distributed without further approval. Boxes with bold text indicate approvals required; italics indicate key prerequisites part of a general GMP assessment.

the aseptic status is verified at critical production steps. The TEBV is now ready for release by a qualified person at both the manufacturer, Cytograft, and the importing organization, Mares Ltd.

Aseptic Manufacturing

Lifeline is an autologous, aseptic and living product and is therefore not sterilized terminally. Since the product is intended for use in the EU, facility requirements are defined in EU GMP Annex 1.

Facility

Annex 1 of EU-GMP prescribes the use of clean room suites including EU grades D-A segregated by air locks and pressure cascades (*i.e.*, a rigid wall facility). The key decision points on the path to such a facility include the choice between outsourcing vs. internal manufacture.

- **Outsourcing involves use of a contract manufacturer, whose facility is already GMP-certified.** This liberates the manufacturer of a significant investment. It does not, however, void the need for validation runs and for a process lasting up to nine months, thus limiting the benefit of this approach. By using the contract manufacturer's personnel, know-how is transferred. Distribution of trade secrets and proprietary techniques in an industry driven more by know-how than patent protection, is an important issue to balance in the "make vs. buy" decision process.
- **Internal manufacture in a purpose-built facility.** This requires setup and initial certification—a significant investment. However, staffed by the manufacturer's own personnel, it provides complete control and results in a tangible asset in terms of both the physical structure and trained technicians.

The principal requirement for a cleanroom facility is the ability to support manufacture at prescribed hygiene levels as principally determined by continuous particle monitoring and intermittent viable particle monitoring. To meet appropriate limits, an air handling system is required to deliver defined quantities of HEPA filtered air to critical zones. The most critical zones are those where manipulations of open product are carried out. These are Class A biological safety cabinets placed in a Class B environment with supportive Class C and D zones. The choices are:

- **Central air handling.** A central air handling system typically allows for greater control over the manipulation of air including, for example, humidity levels. Air is delivered through ducting and HEPA filters to outlets into cleanroom zones. Ducting and outlets are not intended to be moved because fans are removed from outlets, so the system is relatively quiet.
- **Decentralized air handling using individual HEPA fan-filter units.** These provide air directly to clean room zones. Air control is limited to flow rates while a standard air conditioning unit controls temperature. Individual

HEPA filter/fan units are placed in a grid ceiling system providing flexibility regarding the total number of units per area (up to full coverage) and location. Therefore, in addition to the considerations laid out in corresponding ISO guidelines[4] (ISO 14644) filter density and hence, air flow may be selectively increased in critical areas. Subsequent process changes may be factored in by changing the configuration with relative ease. Due to the proximity of fans and outlets, the system is less quiet than the central air handling solution.

With this in mind, Cytograft opted for a dedicated facility. Their process is unlikely to expose cells and tissues to conditions resulting in undue desiccation, so tightly controlling minimum levels of humidity were not a priority. Considering the state of development and the likely need for flexibility in adapting the facility to future needs, Cytograft opted for a grid/HEPA filter/fan unit system and finds it adequate for its purpose.

Raw Materials

Cell-based products typically originate from R&D projects where choice of raw materials is frequently based on parameters other than quality and regulatory compliance. Thus, when converting such a process/product into a development stage appropriate for human application in clinical trials, the choice of raw materials according to quality aspects becomes an urgent need. Perhaps the greatest challenge in this regard are products of animal origin (POAs). In principle, POAs carry the risk of introducing animal pathogens, as highlighted by the EMEA 2004 topic[5] of transmissible spongiform encephalitis (TSE).

- **Serum.** Currently, most cell culture media used for adherent cell culture depend upon bovine serum from fetal or newborn sources which provide factors necessary for successful cell culture. These may range from growth factors (*e.g.*, of the FGF family) to adhesion molecules such as fibronectin. While serum-free media exist and are industrially used for cell lines producing recombinant proteins (*e.g.*, CHO, NS0), cell types supported by such media often grow in suspension or in small clusters. Adherent cells, in contrast, have different requirements and there are few commercial applications using a serum-free media for adherent cell populations. While in principle sera from controlled herds (preferably located in countries considered to have a negligible TSE risk[6]) might suffice. However, EMEA requires a reduction in the levels of infectious agents. This is currently accomplished by γ-irradiation, which can lower the potency of sera and increase the challenges to cell-based therapies in the EU. The European Directorate for the Quality of Medicines (EDQM) provides certificates for sera which satisfy their criteria, and it is useful to select suitable sera from amongst these:
- **Auxiliary agents.** The same logic applies to auxiliary agents such as proteolytic enzymes. Materials are selected in a γ-irradiated quality. Non-animal alternatives may be available such as plant-derived materials like trypsin.
- **Cell culture media.** These are complex mixtures of amino acids, salts, glucose and vitamins. Amino acids, lipids and heparin may be of microbial, human and porcine origin, respectively. Despite sourcing these materials in GMP quality, all media components left in the final product should be viewed as inactive ingredients of the finished product (*i.e.*, excipients) and must be described in detail. Thus, media have to be selected for functionality, for absence of unapprovable ingredients, and be sourced from a vendor who is able to provide complete traceability.

Thus, when developing a cell culture process, ingredients need to be selected that, apart from functionality, satisfy regulatory needs. This can be accomplished by choosing vendors who focus on quality aspects and are able to provide materials in qualities suitable for human use. Despite this, key raw materials such as serum-free media are not widely available. Therefore, bovine serum and other POAs are currently used and the corresponding risk assessment is clearly a key point of regulatory review (Table 1).

TABLE 1. Critical reagents for the Lifeline process.

REAGENT	ISSUE
Proteolytic enzymes	POAs
Growth media	POAs
Bovine serum	POA/Mycoplasma, TSE
Antibiotics	Potentially masking microbial contaminations

TABLE 2. Critical permits for direct marketing in Germany.

TOPIC	PERMIT
Certifying EU importer	GMP certificate for importer
Harvesting serum	Permit acc. to AMG §13
Harvesting biopsies	Permit acc. to AMG §20
Process/plant inspection	Import permit acc. to AMG §72a

TABLE 3. Critical permits for clinical trials in Germany.

TOPIC	PERMIT
Certifying EU importer	GMP certificate
Harvesting serum	Permit acc. to AMG §13
Harvesting biopsies	Permit acc. to AMG §20
Process/plant inspection	Import permit acc. to AMG §72
Institutional review of clinical study	Approval ethics committee
Permission to conduct study	Study permit Paul Ehrlich Institut

In-Process Controls

Due to the duration of the Lifeline manufacturing process, in-process-controls (IPCs) are used to check both functional characteristics and aseptic status:

- **Functional characteristics.** Cytograft uses IPCs to check sheet thickness, sheet strength and burst pressure. Because IPC devices were not readily available commercially, they had to be custom built. During the design phase, it was paramount to integrate the requirements of GMP and validation needs. They had to be reliable, easy to use, and allow for efficient cleaning.

While sheets grow and increase in strength, their thickness increases. This is taken as an indicator for the completeness of the process. Cytograft has engineered a device which uses optical properties to assess sheet thickness non-invasively, providing required data without compromising product. Another key issue, reliable record keeping, was addressed by recording results on paper or by otherwise producing unalterable output.

• **Aseptic status.** This includes tests for endotoxin levels, sterility and mycoplasma. While the first may be completed in a day, the latter two take a minimum of 14 and 28 days, respectively, and therefore the timing of these within the process is important. Specifically, as part of release procedures for a living product, these tests need careful timing to facilitate data availability at the time when product is finished and must be released.

Regulatory Considerations

Cytograft manufactures Lifeline in the USA and intends to import it into Germany for clinical studies. In general, investigative medicinal products (IMPs) manufactured outside of the EU and to be tested in Germany require an import permit along with an importing organization based in the EU. These services, as well as the various interactions with authorities, hospitals, investigators and contract analytical labs are provided by Mares Ltd. To date, Mares Ltd. has received the required permits, features a full set of responsible personnel (QP, Head of Control, and Head of Production) and is GMP-certified. Permits involved in the importing process are:

• **Confirmation of GMP compliance.**

• **Harvest biopsies according to §20 AMG.** Key components are contracts and assessments of these medical facilities, analytical laboratories, and harvesting organizations.

• **Harvest serum according to §13 AMG.**

• **Import according to §72 AMG.** This is equivalent to a manufacturing permit (Herstellerlaubnis) as required for IMPs manufactured in the EU. The permit is obtained through review of the process, facilities and quality system by the authorities of the Land (state) in which the importing entity resides. As the primary expertise of authorities of the Länder (states) is often in classical drug manufacture rather than cell culture based processes, advice may be sought from the Paul Ehrlich Institute (the federal agency for biological medicinal products). From a sponsor's view, this is likely to be a positive as it allows for obtaining input prior to submitting the clinical trial application. However, a potential downside is the time it might take to find a slot for which all parties involved are available, so it is advisable to raise this issue early to commence advanced scheduling.

• **Ethics committee approval.** The ethics committee responsible for the clinical study site(s) will weigh the risk and benefit of the study. Key topics of the application are the protocol, the investigator's brochure, and proof of insurance.

• **Clinical trial approval.** The federal authority—here, the Paul Ehrlich Institute—requires the aformentioned plus the application itself. Key components are the investigational medicinal product dossier (IMPD) and the application form. Describing tissue-engineered products in the context of these documents requires a degree of adjustment in the structure (originally designed with small molecules in mind) to the novel properties of these products.

The Regulatory Landscape

As of December 2008, the Advanced Therapy Medicinal Product (ATMP) regulation applies and regulates the approval of tissue-engineered products. Prior to this, when an import or manufacturing permit was issued according to §72a AMG, such products were put on the market in Germany directly and without further approval. However, in contrast to investigational medicinal products used in the context of clinical trials, follow up is limited to what is considered regular clinical follow up and an import permit (according to §72a AMG) necessitates an on-site inspection.

The regulatory pathway for obtaining approval for clinical trials as well as for direct marketing are depicted in Figure 1. Individual permits required are shown along with key components of these permits. See Tables 2 and 3 also. The importing organization, Mares Ltd. (www.maresltd.eu), has received approval and is ready to import IMPs.

Conclusions

Lifeline is a tissue-engineered blood vessel made from the patient's own tissue and aimed at small-caliber revascularization needs. It is produced in the USA by Cytograft Tissue Engineering, Inc. of Novato, California. The process and facilities have been readied for importing Lifeline as an IMP into the EU, specifically, Germany. We address processing issues and regulatory requirements, as well as the set up of Mares Ltd. Our organization is authorized to serve as legal representative and importing organization of IMPs in Germany for Cytograft and other groups.

The primary regulating partners for this endeavor were the Bezirksregierung Dusseldorf (authority of the Land in which the importing entity resides) and the Paul-Ehrlich Institute (Bundesoberbehörde fuer biologische Arzneimittel). We have found that early contact with both partners is a welcome and beneficial part of the process. This interaction led to final approval through productive criticism. The collaboration has been an asset for Mares Ltd., and on a larger scale, helps in attracting novel technologies to Germany.

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