Briefing Document

Cellular, Tissue, and Gene Therapies Advisory Committee
(formerly Biological Response Modifiers Advisory Committee)

Meeting # 38

Cellular Products for Joint Surface Repair

March 3-4, 2005

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Introduction

This Cellular, Tissue and Gene Therapies Advisory Committee is assembled to provide the Food and Drug Administration (FDA) with insight and perspectives regarding product, preclinical and clinical concerns confronting the development of products for the repair or replacement of articular cartilage defects. The considerations relate specifically to those products that contain living cells and that are administered for correction of articular cartilage defects. The meeting discussion items include manufacturing quality and control, preclinical considerations, and clinical concerns related to both exploratory and confirmatory clinical studies.

At this meeting, no specific products will be discussed or cited for regulatory review purposes. Additionally, no data presented at the meeting will have undergone FDA review for completeness or accuracy. Instead, researchers in the field will present information on some of the issues concerning development of these types of products. Members of the committee will be requested to consider this information and provide a response to FDA questions. While a consensus is desirable, it is not required. Since the field is developing rapidly, FDA anticipates that all opinions are tentative and subject to reconsideration based upon accumulating data.

The treatment of articular cartilage defects is evolving rapidly, both for surgical treatments and for the specific field of cartilage repair products that contain living cells. Hence, the committee is encouraged to anticipate the range of possible products—present and future— that may be investigated clinically for use in cartilage repair, such as

autologous and allogeneic tissue-engineered products as well as products consisting of cells and artificial matrices.

Meeting Goals

This meeting is arranged to accomplish the following goals regarding the development of cellular products for the cartilage repair:

1. To provide FDA with perspectives on the types of manufacturing and preclinical data critical to the initiation and completion of a clinical development program;

2. To provide FDA with perspectives on the major issues in the design, conduct and analyses of exploratory and confirmatory clinical studies;

3. To provide a public forum to discuss the major controversies in developing these products.

Regulatory Background

To initiate clinical studies of investigational cartilage repair products, a sponsor must obtain FDA concurrence, either through review of an Investigational New Drug Application (IND) or a request for Investigational Device Exemption (IDE), depending on whether the investigational product is classified as a biological product or device. The FDA website (www.FDA.gov) contains information and guidance that sponsors may use in order to comply with the IND and/or IDE expectations. Some investigational products may be classified as combination products composed of two or more different regulatory entities (e.g., device-biologic or drug-biologic). Following the collection of sufficient

manufacturing, preclinical, and clinical data, a sponsor may submit an application to
FDA for the manufacture and marketing of a product for joint surface repair. A Biologic
License Application (BLA) is submitted for a biological product and a Premarket
Approval Application (PMA) is used for a new (unique) device. Detailed information
regarding these applications and other types of marketing applications is also available on
the FDA website. Regardless of the regulatory pathway for marketing approval (BLA or
PMA), sponsors must provide similar supportive manufacturing, preclinical, and clinical
data.

Joint Surface Repair—the Clinical Problem

One of the major hurdles to obtaining marketing approval for an investigational product
is the requirement that the sponsor submit clinical data persuasive of the clinical benefit
of the product. The nature and extent of clinical data to be obtained in support of a market
application is one focus for the clinical topics to be discussed at this meeting.

Clinical Studies

Information cited below is intended to provide a brief summary of the most common
terms, procedures and considerations related to articular cartilage repair, especially for
readers with little prior exposure to this field. The attached references provide
substantially more detail.

Pathophysiologic Considerations

In principle, products containing living cells might be used to repair various joints. At
present, nearly all investigational activity has focused on the knee. Accordingly, we
present the following description of this joint and its pathobiology, with the
understanding that many of the considerations to be discussed might be applicable to
other joints as information is accumulated.

The adult knee is a complex joint consisting of the femur, tibia, and patella, as shown in

Figure 1. Also shown are certain ligaments, tendons, and meniscal tissues that serve to

stabilize the knee. The ends of the tibia and femur (the medial and lateral condyles), as

well as the underside of the patella are covered with articular cartilage. Articular cartilage

provides a smooth, resilient surface for joint motion despite repetitive exposure to

pressure loads, shocks, or other mechanical stresses, a limited blood supply, and little or

no potential to regenerate itself effectively.
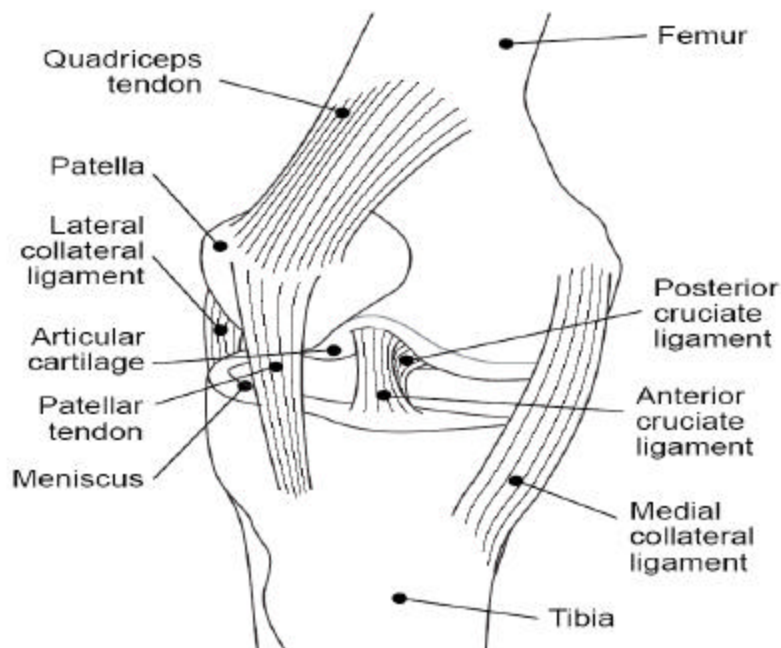
Figure 1. Major Components of the Knee

Articular cartilage consists of chondrocytes and extracellular matrix. Chondrocytes

account for approximately 5% of the tissue volume and the matrix contributes about 95%.

Certain characteristics (such as gross and microscopic appearance) of the matrix allow

cartilage to be categorized into two major categories: hyaline or fibrous cartilage. Hyaline cartilage is distinguished histologically by the homogenous appearance of the matrix, especially when viewed under polarized light, and the round or oval shape of chondrocytes. In contrast, fibrous cartilage is characterized histologically by the presence of bundles of collagen fibers, usually arrayed in an irregular manner when viewed under polarized light. Fibrous cartilage is a form of dense fibrous tissue and accounts for the cartilage found in intervertebral disks, the pubic symphysis, and tendon insertions. The detection of fibrous cartilage in biopsies of knee cartilage is generally thought to be indicative of injury and suboptimal healing. In general, fibrous cartilage does not possess the load-bearing capabilities or resilience of hyaline cartilage.

Hyaline cartilage, the normal articular cartilage of the knee, is a complex mixture of chondrocytes, water, and various extracellular matrix proteins, proteoglycans, and polysaccharides. Chondrocytes are the only normal cellular component of cartilage; the tissue contains no nerve cells or blood vessels. Chondrocytes synthesize and secrete extracellular matrix macromolecules, including collagens, proteoglycans and various noncollagenous proteins. Water normally collects among the extracellular proteins and accounts for approximately 75% of the wet weight of articular cartilage. Most of the water within cartilage is bound loosely within the matrix and exchanges readily with water in synovial fluid. This water exchange allows nutritional support for chondrocytes, a process that is thought to involve both diffusion and mechanical pumping processes associated with joint motion and cartilage compression. This compression—the ability to be indented by a load and to recover from the deformity—is thought to be among the most important functions of articular hyaline cartilage.

Articular cartilage has limited ability to repair itself following trauma. Trauma-induced

cartilage defects that do not penetrate the entire thickness of cartilage (i.e., the

subchondral bone plate) usually remain as defects and, according to some reports, do not

progress in the absence of additional trauma. In these situations, the remaining

chondrocytes appear incapable of replication or generation of sufficient matrix to fill the

defects. In traumatic injury that penetrates the subchondral plate, partial healing may

result from fibroblastic proliferation into the defect, a process that commonly results in

the formation of fibrous cartilage.
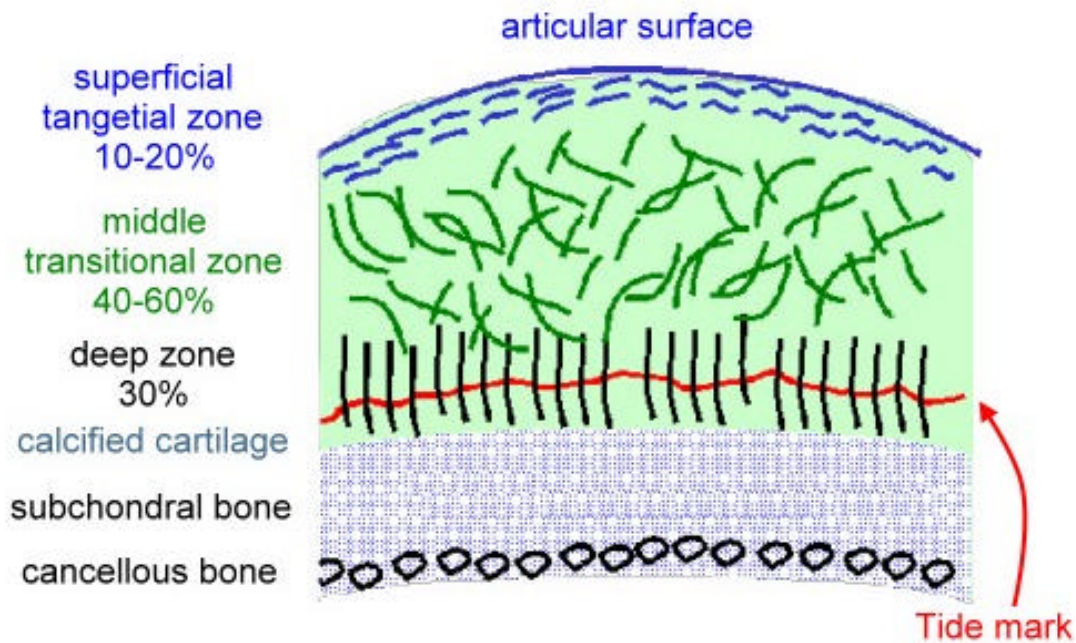
Figure 2. Histological Architecture of the Joint Surface

The clinical symptoms that result from cartilage defects are variable and range from no

discomfort to chronic pain with joint effusions and reduced mobility, depending on

factors such as the size and depth of the lesion and patient activity level. Painful synovitis

may develop in the knee following the release of enzymatic metabolites from the damaged cartilage. This process may culminate in arthritis, further destruction of the cartilage, and the ultimate need for knee replacement.

Chondral defects of the knee are thought to be common. One author reported finding a chondral lesion during 63% of over 31,000 arthroscopic procedures [1]. In a published arthroscopic case series of subjects undergoing ligament surgery, the authors found that the clinical outcomes for patients did not differ between patients who had incidental cartilage defects and those with no defects [2]. This finding supports a general understanding that asymptomatic cartilage defects need not be treated. Nevertheless, the authors of other published reports have expressed the opinion that some cartilage defects will, with time, progress and culminate in immobility [3]. The discrepancy between these two points of view underscores the need for controlled clinical studies in evaluating products used to repair cartilage defects.

Cartilage Repair Options

The chief goal in the treatment of symptomatic cartilage defects is the relief of pain and restoration of function [4]. Current treatments for symptomatic cartilage defects include conservative management with analgesics and anti-inflammatory agents, various rehabilitation programs, and/or surgical intervention. Surgical treatments are developed and modified relatively frequently. The procedures most commonly cited in publications are summarized below. These treatments may be divided among three categories as follows: debridement/lavage of loose or worn articular cartilage, stimulation of a repair process from the subchondral bone, and repair or replacement of the damaged articular surface.

1. Debridement/lavage:

Relatively soon after the introduction of arthroscopy into clinical practice, lavage, a form of rinsing the knee joint, was observed to be associated with symptomatic improvement in some patients with cartilage defects [5]. The combination of lavage with debridement, (the resection of damaged tissue) has been has been reported by some investigators to result in better outcomes than lavage alone[6, 7]. Other investigators have reported the results of a controlled clinical trial in which arthroscopic lavage or debridement produced no benefit compared with a placebo procedure in osteoarthritis of the knee[8]. The pathophysiologic basis for symptomatic improvement reported following lavage/debridement is unknown but has been attributed to the removal of pro-inflammatory tissue. Both debridement and lavage may be accomplished through an arthroscopic procedure.

2. Stimulation Of A Repair Process From Subchondral Bone:

In these procedures, subchondral bone is penetrated surgically to prompt the infusion and filling of the cartilage defect with bone marrow and other cells that are thought to initiate repair or partial regeneration of the joint surface. A carefully designed post-operative rehabilitation program is regarded as a critical component of all these operative treatments. A range of clinical outcomes has been reported for these procedures, but few controlled, prospective clinical studies have been performed. In general, they result in the formation of predominantly fibrous cartilage, not hyaline cartilage.

a. Microfracture:

Microfracture surgery involves the use of specially designed surgical instruments
(awls) to make multiple perforations, or microfractures, into the subchondral bone
plate. The perforations are made as close together as possible within the cartilage
defect but not so close that one perforation penetrates into another one. Preservation
of the subchondral bone plate is regarded as an important part of the procedure.

b. Abrasion:

Abrasion surgery involves the removal of 1 to 2 mm of the exposed bone within a
cartilage defect using a powered burr.

c. Drilling:

Drilling procedures penetrate the subchondral bone within a cartilage defect through
the use of a powered drill. Although the area is infused with saline during the
procedure to cool the tissue, the heat exposure associated with some burrs or drills
has been proposed as a factor in suboptimal cartilage repair.

1. Repair or replacement of the articular surface:

These surgical procedures consist of various approaches to cover the surface of the
cartilage defect with either autologous or allogeneic tissue. Variable clinical
outcomes have been reported for these procedures and most of them are thought to
result, with a few exceptions, in either a mixture of hyaline and fibrous cartilage or
predominantly fibrous cartilage. These types of procedures are generally preferred for
larger cartilage defects.

a. Mosaicplasty:

Autologous osteochondral mosaicplasty involves the excision of small osteochondral cylinders from a "minimally weight bearing" portion of the femoral condyles followed by implantation of these cylinders within the cartilage defect. Major considerations in the use of mosaicplasty include the size of the cartilage defect and the amount of tissue available from the minimally weight bearing portions of the knee.

b. Tissue grafts:

Allogeneic or autologous osteochondral tissue grafts may be implanted within a cartilage defect. Allogeneic grafts involve the use of fresh, viable cartilage (which survives) and dead bone (which is replaced by host bone). The technical difficulties of these procedures, combined with the infectious risks associated with allogeneic tissue, have limited their use.

Both autologous periosteal patch and perichondral surface grafts have been used in some patients with cartilage defects. However, the clinical experience with these procedures is limited and reports of success of the procedures have varied considerably.

c. Autologous chondrocyte implantation:

In this procedure, autologous cartilage tissue is harvested, the tissue dissociated in a laboratory, and isolated chondrocytes expanded in number. Subsequently, the expanded chondrocytes are implanted within the cartilage defect, with the cells held in place by a periosteal graft. At least two surgical procedures are needed, one for harvesting the cartilage and another, usually a few weeks later, for implantation of the expanded chondrocytes. One autologous chondrocyte product is currently licensed by

FDA for clinical use as a treatment for patients who have failed a prior surgical procedure that was intended to correct the cartilage defect.

Clinical Study Considerations

Design:

During the development of a cartilage repair product, clinical studies may be broadly divided into two categories: exploratory or confirmatory. Exploratory clinical studies, the earliest clinical studies in product development, examine the safety and bioactivity of the product, evaluate dose-response effects, and thus provide information critical to the design of subsequent confirmatory studies. Exploratory clinical studies help generate a specific hypothesis that is then tested definitively in the confirmatory clinical study.

Options for the design of exploratory clinical studies are broad and largely dependent upon the study objectives. For example, an uncontrolled study may suffice to detect major safety concerns associated with the first administration of the product to human subjects. On the other hand, a controlled study design may be necessary to detect important pilot safety and treatment effects if the effects occur commonly during the natural history of the underlying condition. Confirmatory clinical studies must use a control group to assess the safety and/or efficacy of a product definitively. In general, the use of a concurrent (as opposed to historical) control is thought to be especially important for clinical studies that evaluate relatively subjective endpoints such as changes in knee pain or function. Hence, a controlled clinical study design is commonly cited as especially important for confirmatory clinical studies of products

used in the repair of cartilage [3]. The choice of the control product or treatment is one of the major challenges in the design of these studies and a broad range of opinions have been expressed about the role of a placebo control, any specific surgical procedure as a control, and the use of subjects undergoing a variety of surgical procedures as a single control group.

Various other considerations are important for confirmatory clinical studies of cartilage repair products, such as the need for standardization of surgical techniques, concomitant treatments, and rehabilitation programs. Additionally, the duration of follow-up and the types of follow-up evaluations are important issues for a sponsor to consider in the design of confirmatory clinical studies of cartilage repair products.

The ability to perform controlled clinical studies in the evaluation of articular cartilage repair products has, at times, been regarded as difficult or impossible. Attached is a publication that illustrates many of the challenges associated with the conduct of a controlled study. This study is also notable for its use of a variety of potentially important outcome measures (Knutsen, et.al, 2004; attached).

Clinical Outcomes:

The committee will be asked to discuss the importance of a number of clinical study outcomes. The most commonly cited clinical outcomes regarded as important for cartilage repair products and procedures include measures of changes in patient symptoms, such as knee pain and function. Surrogate endpoints evaluating gross or microscopic elements of knee structure have also been proposed.

a. Patient symptoms and knee function:

Changes in patient symptoms in clinical studies may be evaluated with a variety of methods, e.g., changes in pain medication usage, changes detected through the use of patient and/or investigator questionnaires, or specific types of scales, such as visual analogue scales of pain. In practice, questionnaires are among the most commonly cited measures of changes in symptoms and knee function.

Many questionnaires and knee-rating scales have been developed and tested in clinical studies or clinical practice[9-11]. The most commonly cited measures include:

- the Lysholm scale

- the Cincinnati Knee-Rating System

- the American Academy of Orthopaedic Surgeons (AAOS) Sports Knee-Rating Scale

- Activities of Daily Living of the Knee Outcome Survey

- Short Form-36 (SF-36), or a subset, the SF-12

- Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index

- International Knee Documentation Committee (IKDC) Subjective Knee Evaluation

- Knee Injury and Osteoarthritis Outcome Score (KOOS)

Several of these scales and questionnaires consist of multiple components, only some of which relate directly to knee symptoms and/or function. The clinical usefulness of each of these scales and/or questionnaires is subject to many considerations, including the extent of prior clinical data supporting the measure and the incremental sensitivity of the measure in detecting important changes. Studies of reliability, validity and responsiveness of several of the scales have been published [9-11]. Additionally, the

usefulness of any specific measure may depend on specific study-design considerations, such as blinding, evaluation time points, type of injury, method of treatment, and subject characteristics.

Other measures of potential importance in the assessment of knee function include joint range of motion and physical examination findings (strength, alignment, ligament laxity, etc).

b. Cartilage structure:

Many published clinical studies have described, as an important outcome, the extent of cartilage structural integrity following administration of a cartilage repair product or the performance of a repair procedure. In general, these assessments have consisted predominantly of "second-look" arthroscopy (with or without biopsy) and various imaging modalities.

Arthroscopic evaluation of cartilage repair products has, in many published clinical reports, consisted of visual examination of the joint surface and histopathologic analysis of biopsies. The cartilage surface is readily visualized and a scoring system has been used to grade the findings (ICRS, International Cartilage Repair Society macroscopic score). Gross and microscopic findings consistent with hyaline cartilage are regarded as preferable to appearance of fibrous cartilage or a combination of fibrous and hyaline cartilage.

One of the major limitations of arthroscopic evaluation is the invasive nature of the procedure. Histologic analysis provides direct information regarding the nature of tissue contained in the repaired defect. On the other hand, whether the gross or microscopic

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appearance of a joint surface that has been treated with an investigational therapy gives an accurate, clinically relevant indication of compressibility, load bearing, or other functional characteristics is unknown. It is thus appropriate to weigh the information to be gained from these approaches against the potential morbidity that may be incurred.

The most commonly cited imaging studies of cartilage include standard radiography and magnetic resonance imaging (MRI). Standard radiography has very little utility in the assessment of cartilage defects because of the focal nature of the defects. On the other hand, MRI provides much greater information regarding cartilage integrity and is frequently cited as the most useful noninvasive method of cartilage imaging [12]. Many technical details relate to successful MRI imaging of cartilage and the utility of MRI imaging may vary considerably from center to center based upon variations in the performance of the procedure.

Clinical Questions:

1. Confirmatory clinical studies are controlled studies designed to test hypotheses generated from the exploratory clinical studies. They should provide definitive information for licensure or marketing approval. The primary endpoint for a confirmatory clinical study should be a clear, meaningful measure of clinical benefit. Please discuss the extent to which each of the endpoints listed below meet this need. Please cite any other endpoints you regard as important for confirmatory clinical studies. Please note that the list includes some endpoints that have been regarded as clearly clinically meaningful measures and others that have been regarded as useful but not definitive measures of clinical benefit.

   a. Changes in knee function as measured by scoring systems such as the WOMAC function score.

   b. Changes in pain as measured by scoring systems that take medication usage into account.

c. Changes in clinical examination findings (e.g., range of motion, patient's global assessment, etc.).

d. Changes in the appearance of the joint surface on arthroscopy and histopathological appearance of a biopsy sample from the treated site. Please also discuss whether the potential morbidity entailed by biopsy outweighs its utility as an endpoint.

e. Changes in the appearance of the joint surface and joint space on Magnetic Resonance Imaging or other noninvasive techniques (e.g., X-ray, computerized tomography, etc.).

2. Confirmatory clinical studies should provide robust, verifiable evidence of the clinical benefit afforded by a cartilage repair product. Please discuss the importance and limitations of the following aspects of clinical study design for sponsors to consider when designing confirmatory clinical studies. Please highlight those situations where flexibility may be acceptable and identify any ancillary considerations that might optimize the clinical study design.

a. The nature of the control group; for example, active product or active dose comparator, surgical procedure comparator, historical comparator, etc.

b. The importance of blinding procedures; for example, complete blinding versus the use of blinded evaluators or other options.

c. The duration of the clinical studies, as it relates to assessing short term as well as long term benefit in time weighted or landmark analyses. Specifically, at what time points should important endpoints be evaluated in order to assess the success and durability of a treatment effect?

Preclinical Studies

Prior to initiation of human trials, several types of information must be gathered in non-clinical models. Classical "small molecule" drug development programs have focused on pharmacology and toxicology studies, but other kinds of data are also important, especially when relatively little is known about the entire class of experimental products under investigation. Studies to provide a scientific and medical rationale for evaluating the experimental product in humans, to support an initial human dose, and to evaluate potential toxicities are well-accepted components of most pharmaceutical development programs. Due to their inherent complexity, products containing cells require substantial additional information. These data may be gathered through numerous different studies, including the following assessments: 1) interactions between cellular and device components of a combination product; 2) biocompatibility analysis of the device component; 3) analysis of the contributions of different components of a product to its biological action; 4) evaluation of potential immune responses to the product; 5) exploration of potential clinical or surrogate endpoints; and 6) qualification of analytical tests used during manufacture of the product and for lot release. It is therefore reasonable to expect that various experimental systems would be combined to assemble a data set sufficiently comprehensive to allow sound decisions regarding conduct of initial clinical studies. The models selected should reflect the type of information needed for the product in question.

One of the most basic requirements in any pharmaceutical development program is data to provide reasonable assurance of the product's safety. In addition to conventional safety studies that assess potential toxicities in a context designed to model the clinical

indication, many cellular products need to be evaluated for their potential to undergo unanticipated undesired changes in their characteristics, such as malignant transformation. Models designed to address this concern should evaluate a number of cells sufficient to detect rare events with reasonable statistical confidence. It is also important to assess the potential for adverse events in models approximating the human clinical situation. Frequently, this information can be gained in the same studies used to confirm the scientific and medical rationale for the approach ("proof-of-concept" studies). These studies can also be used to collect information on the physiological disposition of the study agent. For combination products, routine device biocompatibility testing and testing of the biocompatibility of the device/matrix component with the cellular component may also be incorporated, if appropriate. Regardless of whether a disease model or healthy animals are used, studies intended primarily to provide safety data are termed "pivotal toxicology studies".

Proof-of-concept studies should mimic the intended clinical indication as closely as possible. This is needed not only to allow the most reliable evaluation of the therapeutic potential of the experimental product, but also to assess the likely duration of clinical effect. The latter consideration is of special importance, because given the risks inherent in any cellular therapy, failure of the intervention after a brief interval of benefit could be viewed either as a late-occurring toxicity or a treatment failure. For joint lesions, this presents special problems, because the model needs to resemble a human patient not only in applicable cell biology and pathophysiology, but also in joint mechanics and anatomy.

Animal studies may also be used to evaluate specific characteristics of an experimental product. An example relevant to the repair of joint surfaces is the use of immunodeficient

rodents to test whether a product candidate is capable of forming stable articular cartilage *in vivo*. While not addressing conventional issues of safety or proof-of-concept per se, such experiments may provide valuable, or even crucial insight into critical parameters for product manufacture or the suitability of potential release tests.

Animal models of joint surface defects

Anatomic and mechanical considerations inherent to the microenvironment of the articular surface in which the product is implanted are important potential determinants of the activity and safety profiles[13-15] of cellular or combination products for articular cartilage repair and regeneration. For example, preexisting instructive factors present in the native microenvironment can influence the *in situ* differentiation of cellular products[13, 16, 17]. Conversely, exogenous cells may have the capacity to alter proliferation and differentiation of the patient's cells. In addition, mechanical forces such as static loading from standing and dynamic loading from locomotion greatly affect the local microenvironment at the articular surface and have been shown to influence the growth and differentiation potential of cultured cells[18-20] and the *in vivo* durability of products intended for cartilage repair. Various species differ markedly with respect to extent of cellularity, extracellular matrix, and overall organization[21]. These additional interspecies differences in microenvironment and cell biology may also limit inferences that can be made regarding the eventual clinical performance of these products.

The human articular cartilage injury most frequently considered for treatment with cellular therapy is a partial thickness injury that does not extend to the subchondral bone. The characteristics (i.e., size, shape, and depth of the experimental defect) in the animal model should mirror the dimensions of the clinical defects as closely as possible. The

degree to which this is achievable depends in part on the critical size defect (CSD), which is the smallest defect in the native cartilage that will not heal without intervention. When injuries exceed the human CSD, they are unlikely to heal naturally, and are therefore appropriate targets for therapeutic intervention. Such injuries are modeled in animals by attempting to create partial thickness lesions. This is done by removing the superficial portion of cartilage (above the tidemark) while preserving an intact barrier to the underlying subchondral bone and marrow. In practice this has proven to be difficult technically. Penetration or microfracture of the subchondral bone, either prior to or immediately after placement of an experimental product, allows cellular components of the native marrow to compete with the experimental product for healing of the lesion. This frequently results in fibrocartilaginous repair in both control (untreated) and treated lesions, which may preclude unambiguous evaluation of the therapeutic effect of the product itself. In fact, penetration of the subchondral bone essentially produces a model of the surgical cartilage repair procedure known as microfracture, as described in the previous section. Consequently, the depth of native cartilage is a key factor in animal species selection. The size of the CSD varies by species (Table 1), as does the thickness of the articular cartilage. Useful disease models should thus afford adequate cartilage thickness and an appropriate CSD.

Review of the published literature reveals that numerous joints in several different animal species have been used to evaluate cartilage repair products [15, 22, 23](Table 1). The stifle, which is the quadruped joint resembling the human knee joint most closely, has been the most frequently reported in the literature. Species diversity is also reflected in differences in articular anatomy, cartilage thickness, cartilage histology, cell biology, and

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age at skeletal maturity (Table 1). Within the stifle there are several articular surfaces available for experimentation: the femoral condyle, the trochlear groove, and the patella, which articulates with the trochlear groove and therefore has also been used as a potential test site. The degree of loading (both static and dynamic force) on the articular areas varies due to species-specific intraarticular anatomy and mechanics. Therefore the most common site of clinical damage, the femoral condyle, is not always the appropriate site to use in the animal to mimic the intended clinical indication. For example, in a commonly used animal species such as the goat, a periosteal flap used to secure the cellular product in the defect may fail due to the inherent loading forces on the femoral condyle and therefore compromise the activity of the cellular product. This inherent diversity among various animal models thus also secondarily influences the choice of experimental defect site, the study duration, and the depth and cross-sectional area of experimental cartilage defect available for testing. For example, the area of the articular surface available for defect creation and product implantation is related not solely to articular size, but also to the additional features such as condylar curvature and weight-bearing characteristics. In the case of testing cellular products these constraints also may limit the size of the product that can be produced.

Small Animal Models

The rabbit, which is the most commonly used small animal species in joint repair, has the advantages of lower cost and relatively early skeletal maturation (approximately nine months), as compared to the large animal species that have been used in this field (see Table 1)[22, 24, 25]. All four major articular surfaces of the stifle (femoral condyle, trochlear groove, tibial plateau, patella) of the rabbit have been reported in

the literature[26, 27]. The relatively small area of the rabbit's articular surface and depth of articular cartilage restricts the size of the defect that can be created and therefore the volume (dose) of cellular implant that can be tested. In addition, the small size of the articular surface limits the type of potential device components that can be tested in the rabbit for fixation of cartilage repair product. For example, full size (clinical) versions of screws and staples cannot be tested within the rabbit stifle. Another concern is that the rate and degree of repair of cartilage damage in the adult rabbit is typically more rapid and more complete than that seen in adult large animals or humans. It has been hypothesized that this is a consequence of the rabbit's relatively high metabolic rate, the availability of relatively large numbers of pluripotent stem cells in proximity to the articular surface, and the relatively small volume of defect that can be created. Additionally the relatively light body weight of the rabbit decreases its utility as a model to test the durability of cartilage repair products. The maximal duration for a rabbit study is typically only six to eight weeks. Thus many interventions that appeared to show great promise in leporine models, as evidenced by complete reconstitution of normal appearing articular cartilage after fairly brief periods, were not useful in other systems. The rabbit has thus been used primarily as an *in vivo* model in the early stages of product development to evaluate biocompatibility, and screen various biomaterials for potential applicability as a scaffold/matrix, as well as to explore basic product design issues of the scaffold/matrix components of combination products.

Large Animal Models

The dog, pig, sheep, goat, and horse have all been used as large animal models of cartilage repair. In general, the size of the stifle increases in proportion to the size of the animal. Therefore, the larger animals allow for the testing of cellular products and associated attachment devices that more closely approximate the size and design of the intended clinical product. However larger animals also require significantly longer to reach skeletal maturity than smaller animals. Skeletal maturity is coincident with microenvironmental changes in the articular cartilage that strongly influence the overall repair process. The cartilage of all adult animals is more resistant to repair than immature animals, and therefore more similar to adult human subjects. Therefore therapies intended to treat injuries in adult humans should be evaluated in animals that have reached skeletal maturity.

The combination of overall increased stifle size, less effective native cartilage repair, and longer lifespan are advantages of large animal models of human clinical indications. The two primary benefits of large animals are the ability to model a clinically useful duration of response (durability) to products more closely and the potential to incorporate minimally invasive or non-invasive endpoints into a product development strategy prior to clinical trials. As mentioned previously, one key requirement for successful implementation of cell-based therapies for joint surface repair is durability of clinical benefit. Due to the biology of cartilage repair in large animals, studies of eight to twelve weeks duration (maximal length in rabbits) are only adequate to provide information on the biocompatibility and early cellular viability in larger animals. Longer-term studies of at least six to twelve months duration are needed to assess the true success of cartilage repair. This study duration

is similar to what is generally thought by the orthopedic community to be needed for initial clinical indications of activity in humans. Additionally, some have attempted to model clinical rehabilitation regimes such as immediate post-operative rest and continuous passive motion can be modeled to some extent in large animal models of cartilage repair[28]. The ability to use large animals to test not just the cartilage repair product, but also the feasibility of various diagnostic modalities such as imaging, biomechanical tests, arthroscopy, and arthroscopic biopsy for the *in situ* evaluation of the product could prove to be beneficial in an overall product development scheme that includes these modalities in clinical trial design.

Canine and Porcine Models

Of the large animal species, dogs and pigs are used infrequently for the testing of cellular products for cartilage repair[15, 29]. The most commonly used surfaces in these two species are the femoral condyle and the trochlear groove, although the patella has been reported as a target site in the canine models. The pig stifle joint angle is unusual because it has a reduced range of motion relative to other quadruped models[30]. The intermediate size of the stifle in dogs and unusual anatomy in the pig suggest that these animals may be most effective in providing a bridge from initial product materials and *in situ* biocompatibility testing in rabbits to more extensive *in vivo* testing in sheep, goats or horses.

Caprine and Ovine Models

Some investigators have also made extensive use of sheep (whose stifle resembles that of goats in many respects), but by far the most frequently used large animal in

cartilage repair studies is the goat [16, 31-34]. The femoral condyle and trochlear groove in the goat are the most frequently used articular surfaces, but some investigators have also used the tibial plateau, the articular surface that opposes and articulates with the femoral condyle. In addition, the patella or lateral femoral condyle of the goat has been used as a non-weight bearing site for product implantation or harvest of source material for autologous cellular products[34]. The popularity of the goat as a model appears to be due to a combination of 1) reasonable cartilage thickness, 2) relatively large stifle size, and 3) ease of use, cost, and availability. Unfortunately, although the stifle is relatively large, the anatomy of the joint is not conducive to routine arthroscopic examination.

Equine Models

In the horse model, the femoral lateral trochlear ridge and femoral condyle have been used most frequently as test sites. The size of the horse's articular surfaces and depth of the chondral plate provide an animal model that resembles humans most closely. The equine model thus allows for large, clinically relevant defects as well as multiple defects per stifle[23, 35, 36], thereby facilitating the testing of clinically relevant volumes/amounts/ doses of the cellular product in defects of the size that occur in humans. Unlike the other animals models used in cartilage repair, the size of the stifle in horses also is sufficient to allow routine arthroscopy for interim visualization of the joint surface and biopsy during a long-term study without sacrificing the animal. This diagnostic modality could thus be correlated with clinical endpoints prior to conducting a human clinical trial[37-39]. In addition, the ability to conduct arthroscopy in a horse model allows the testing of arthroscopic placement of

experimental products in cartilage lesions, which would permit direct evaluation of

this method for the delivery of products for joint surface repair prior to use in

humans.

Table 1. Animal Models used in the assessment of cartilage repair

Species	Breed	Age at Skeletal Maturity (years)	Weight at Skeletal Maturity (kg)	Defect Commonly Used	Cartilage Thickness at Femoral Condyle (mm)	Critical Size Defect (mm)
Rabbit (Leporine)	New Zealand White	0.75	3-4	FC, TG, TP, P	0.25-0.75	3
Dog (Canine)	Mixed, Beagle	1-2	15-30	FC, TG, P	1.3	
Pig (Porcine)	Minipig	0.8-1	20-40	FC, TG		
Sheep (Ovine)	Suffolk, Texel	2-3	35-80	FC, TG	1.7	7
Goat (Caprine)	Dairy, Boer Cross, Spanish	2-3	40-70	FC,TG,TP,P	1.5-2	
Horse (Equine)	Mixed, Thoroughbred, Quarter Horse	2-4	400-500	FC, TG, RC	2-3	9
Human	N/A	16-21	70	FC	2-3	

FC- femoral condyle
TG trochlear grove
TP- tibial plateau
P- patella
RC- radial carpal

Immunological Considerations

Though the articular space is thought to be an area of relative immune privilege in both

animals and humans due to the relative lack of local microvasculature and relative

hypocellularity of articular cartilage and synovial fluid that bathes the articular surface,

rheumatoid arthritis and other inflammatory arthritides suggest that this is not absolute, and therefore immune response to both cellular and device components of combination products (see above) may occur in the articular space. The testing of cellular products derived from human cells in animal models thus poses a special concern, as these cells are xenogeneic to all animal species, and therefore at risk for xenotransplant rejection.

Immunological reactions to human product in animals often necessitate that preclinical studies be performed with animal cellular products that are analogous to the intended clinical product, rather than the actual human product. The determination that a specific animal cell is analogous to the intended clinical product is made on the basis of some combination of morphology, biochemical or molecular biological characteristics, ontogeny, and function. Ideally this determination would be multifaceted and involve not just *in vitro* measures of cell identity, but also incorporate detailed understanding of the *in vivo* activity of both the animal analog and putative human correlate cell. This approach is similar to what is frequently done during preclinical testing of monoclonal antibodies directed against epitopes expressed only in humans. In this situation, an immune response or lack of an applicable epitope limit the ability of the model to evaluate the clinical product. Implicit in the use of analogous animal cells as a means to assess biological activity and/or safety of a human cellular clinical product that is composed at least in part of human cells is the assumption that cells from the two species will respond similarly to the stresses imposed in the *in vivo* articular environment. The data obtained from testing analogous animal cells will provide a partial basis from which to make a risk/benefit analysis that is integral to review of preclinical data prior to initiation of clinical trials. The degree of understanding of the relationship between an

animal cell and its human correlate is an important factor in determining the strength of the extrapolations from findings in animals to the potential risks in humans. The degree to which inherent interspecies biological differences may limit inferences that can be drawn between non-human and clinical studies is not yet known This underscores the importance of additional work to characterize the differences and similarities between species used for preclinical models and humans.

Potential Future Directions

More detailed study of the comparative developmental biology of human and important animal models is likely to uncover characteristics common to the relevant species. For example, though details of terminal differentiation pathways may differ, it is probable that at least some key signal transduction pathways known to be highly conserved phylogenetically will function analogously between species, allowing detailed evaluation of biological responses, both *in vitro* and *in vivo*. These data, in turn, may provide a basis for developing more refined release tests and process controls than exist currently. *In vivo* data from the most appropriate animal models is likely to be of critical importance in evaluating the usefulness of such tests.

Preclinical Questions

3. Please discuss the limitations and capabilities of available animal models for predicting safety and clinical activity, focusing on the following:
    a. How should questions of dose and allometric scaling (i.e., size and shape of animal joint versus size and shape of human joint) be explored in animal models?
    b. To what extent do differences between human versus animal anatomy and cell physiology need to be addressed in an animal model that uses analogous animal cells to model human chondrocyte function? Which specific interspecies differences affect the types of conclusions that can be drawn from animal studies?

c. Are non-invasive imaging modalities such as ultrasound, CT, or MRI adequate as a replacement for interim sacrifices in long-term (six to eighteen month) studies to evaluate for intraarticular toxicity and /or cartilage formation?

d. What role should biomechanical tests play in analysis of cartilage repair in animal models?

e. What role should arthroscopic biopsy play in analysis of cartilage repair in animal models?

f. Are tumorigenicity studies needed for cultured chondrocyte cellular products?

4. Please provide specific comments on the following with respect to a "pivotal" animal toxicology study that is designed to support a clinical trial of a cellular cartilage repair product?

a. What animal model(s) and study duration are needed to support exploratory clinical trials?

b. What animal model(s) and study duration are needed to support a licensing application?

c. Traditionally, *in vivo* toxicology studies include measures of systemic toxicity such as clinical pathology tests and histopathology of major organs. Is this approach warranted for toxicology studies with the following categories of products:

   i. cellular products?

   ii. modified cellular products that may secrete molecules capable of producing systemic toxicities (e.g., *ex vivo* gene therapy)?

5. For an allogeneic cellular product for articular repair, what, if any, additional safety concerns beyond those posed by an autologous product should be addressed in an *in vivo* study prior to initiation of clinical trials?

Product Characterization and Testing

During the development of a product from the exploratory investigational phase through marketing approval, much needs to be learned about the product and critical aspects of its manufacture. The initial focus is on information to support a rationale for human experimentation and to provide reasonable assurance that human subjects will not be placed at unreasonable risk. The immediate application of this data is to allow exploratory safety studies in humans. As development of a product proceeds, additional experience with the manufacturing process and ongoing characterization studies provide more detailed information about the product itself and key parameters in its manufacture. Ultimately, enough is learned to develop an overall strategy to ensure product quality and consistency.

Product reliability and consistency depend primarily on three elements: control of source materials, control of the manufacturing process, and meaningful release tests. Somatic cell therapy products present special challenges in each of these areas. The source material for cellular products for cartilage repair may be tissue obtained by biopsy of the subject or an allogeneic donor. Typically, cartilage from the joint to be treated is used, but other tissues and anatomic sites have been considered. Substantial inherent biological variability is thus unavoidable. Much remains to be learned about how to manufacture cells with clinically useful properties. Cellular products are much more complex biochemically than other drugs or biologics, and the capabilities of conventional analytical methods are limited. The source material, manufacturing intermediates, and the final product may all have very short shelf lives, imposing the further constraint that testing methods must be rapid.

To meet this challenge, detailed studies to explore which characteristics are important determinants of product safety or effectiveness should begin as early as possible. As product development proceeds, the analytical modalities that might best be used to evaluate the product at intermediate stages of manufacture (in-process testing) and to release the product for clinical use (specifications) can be identified. Both types of testing play an essential role in optimizing the entire manufacturing process and ensuring that a quality product will be produced consistently. This level of control is needed to support reliable conclusions about the clinical effectiveness of the product, and is therefore essential for marketing approval.

Tissue source

The majority of cell products intended to repair joint surfaces have been derived from autologous articular cartilage, though recent data suggest that other tissue sources (e.g., periosteum, synovium) might also be considered [40, 41]. The tissue chosen for preparation of the cellular product is perhaps the most critical of the starting materials; it is thus important that acceptance criteria for such tissue be established. Some criteria may be apparent at the time of the biopsy procedure. For example, anatomic site, pathologic involvement of the tissue, and overall condition of the patient—systemic disease, age, etc.—may all affect the quality of the final product. In addition, other characteristics of the tissue measurable by laboratory procedures (e.g., histological analysis, biochemical, measures of gene expression, immunassays, etc.) at the start of manufacture might be useful in determining whether a particular tissue sample will be acceptable for production of a safe and efficacious cellular product.

Specifications and process controls

Reliable approaches to characterization of conventional 'small molecule' drugs are now well understood, and contemporary methods are sufficient for most protein therapeutics. In contrast, analytical techniques currently available for characterizing products with the complexity of living cells or cell populations fall far short of meeting standards expected for the former two classes of products.

Early approaches to characterization of cellular products were often limited to visual assessment of morphology, cell count, and assessment of viability based on exclusion of a vital dye. These methods are inadequate to identify many cell types unambiguously, do not accurately reflect the capability of cellular products to adopt desirable characteristics *in vivo*, and are very insensitive to impurities in the cell population or to significant impairment of cellular metabolism (see below). More recent approaches have included evaluation of cell surface antigens by immunofluorescence microscopy or flow cytometry, assessment of characteristic mRNAs using RT-PCR or microarray, various biochemical analyses, and release of specific bioactive molecules or other functional assays. To date, no publications have appeared that describe use of these approaches to identify characteristics that predict reliable performance of cellular therapies in joint repair.

This problem is compounded for cells used for repair of joint surfaces, because the characteristics they adopt during culture are often very different from those of normal articular cartilage. Thus, the customary focus on characteristics—morphological, molecular, or functional—commonly associated with the desired terminally differentiated tissue has limited utility. Instead, exploratory studies may be necessary to identify other

product characteristics, some of which may not yet be known, that are maintained or developed during product manufacture and are sufficient to ensure the desired clinical benefit. Conversely, the conditions used to culture and expand many of these products are so different from the *in vivo* situation that they may promote the appearance of undesired cell types (e.g., inappropriately differentiated or transformed cells) or other product properties (extracellular matrix components or cell surface antigens associated with pathologic states, etc.). Sensitive assays to detect such potential impurities will therefore also be important.

It is generally assumed that for cultured chondrocytes to be effective in cartilage repair they should express type II collagen and aggrecan but not type I or type X collagen. Type II collagen and aggrecan proteoglycan are the major matrix components of articular cartilage. Conversely, type I and X collagens are not normally expressed by articular chondrocytes and are indicative of de-differentiation and terminal differentiation, respectively. However, it is by no means clear that evaluation of the final product for these markers suffices to provide assurance of satisfactory product performance *in vivo*. It is likely that more comprehensive studies will be required to identify sets of characteristics that can be used to identify cells that will form clinically useful articular cartilage.

Combination products, or products in which cells have organized into structures resembling formed tissues, may require special types of studies. Many approaches already in use for evaluation of conventional medical devices can be applied without modification to artificial matrix components. When cells are embedded in a natural or artificial matrix, it may be necessary to sample portions of a product for testing that may

be destructive. In some cases, destructive and nondestructive test methods could be compared during characterization studies to determine whether the nondestructive test is sufficient during routine manufacturing operations. Whether any forms of mechanical testing for certain products may be appropriate remains to be determined. Another possible complexity for such products is that their properties may vary across three-dimensional space. Thus, differences in spatial distribution (e.g., in gene expression, histology, immunohistochemical markers, etc.) may need to be assessed.

In addition to tests that will evaluate identity and purity, it is important to assess the biological activity, or potency, of the product. The ideal potency assay should measure a relevant biological activity of the product quantitatively. Suitable assays for potency are often particularly challenging to develop. For example, methods based primarily on cell count and exclusion of vital dye give no assurance that a cellular product will perform as intended *in vivo*. This approach does not address any characteristic of the product related specifically to its ability to reconstitute a joint surface. Moreover, dye exclusion is often insensitive to substantial disturbances in cell function and may not be applicable to products in which cells are surrounded by naturally or artificial matrix impermeable to most dyes. For other types of products, alternative assays, including oxygen uptake and ATP content (Hering, B.J. and Pappas, C., *personal communication*) show significant promise. Dyes sensitive to various aspects of cellular metabolism, or more detailed analyses of high energy phosphoryl compounds by mass spectrometry [42] or ^{31}P NMR [43] might also be applicable to cellular products for joint repair.

Historically, animal studies have been used to assess potency in cases where *in vitro* assays were problematic. As noted previously, the short shelf life of cellular products for

cartilage repair may limit the practicality of this approach. A useful compromise would be a set of tests that would be feasible in a manufacturing environment that could be qualified by using performance in one or more animal models as a 'gold standard'.

In summary, some product characteristics that can both be measured prior to administration and also predict beneficial or harmful behavior *in vivo* may be known; others may not be apparent *a priori*. Characterization studies to address this question are therefore of special importance. These studies might be facilitated by, or even require, additional insights into the biology of cartilage specification and determination in humans and model organisms together with the application of novel analytical techniques, some of which might not be practical in a manufacturing environment, but could help identify suitable methods.

One requirement common to all such studies is a means to evaluate the influence of various product characteristics on performance *in vivo*. One of the most promising approaches is to relate product characteristics to performance in one or more preclinical models, as discussed above for potency. Whether or not this will prove practical depends on the capabilities and limitations of these models, as discussed earlier. Careful work to explore the relationship between analytical tests suitable for use in a manufacturing environment and performance of experimental products in various model animals and humans is thus likely to be an important step on the critical path toward development of cellular products for joint repair.

Product Questions

6. Characteristics of the starting tissue, which could be derived from the involved joint or a different site, may influence the quality of the cellular product substantially. Please discuss

what criteria should be used for obtaining such tissue (e.g., anatomic site, pathologic involvement of the tissue to be collected, etc.). Please discuss the gross characteristics that would be useful, including those that may be visible at operation before excising the biopsy, and/or microscopic or molecular characteristics evaluated following collection but before use.

7. Noting that cells intended for repair of joint surfaces, when grown *in vitro,* may not express characteristics of cells that produce differentiated cartilage, please consider the following question: What characteristics (e.g., based on analysis of proteins, extracellular matrix components, mRNA expression levels, cell surface antigens, cellular morphology, functional properties, or other parameters) could be used to identify cells that will form stable chondrocytes *in vivo*? Where appropriate, discuss characteristics that should be absent from these products as well as those that should be present.

8. For licensed biological products, each lot of final product must be tested for identity, purity, and potency prior to clinical use. Please discuss what analytical tests and acceptance criteria could be applied to each of these parameters to provide reasonable assurance of adequate product performance *in vivo*.

   a. Please identify the characteristics discussed under question 7 that would be useful in developing such tests.

   b. Given the capabilities and limitations of animal models discussed previously (Question 3), please discuss how these models may be used to provide data to support the *in vitro* characterization tests.

   c. Please discuss biological activity assays that may be used to measure the potency of each product lot to ensure product consistency. Are methods based on determination of viable cells by dye exclusion (e.g., for cells used immediately after culture) or formation of colonies in soft agar (where time permits, e.g., if final product is cryopreserved) adequate? If not, please suggest appropriate alternatives.

9. Many products in this category consist of cells within a biological or artificial matrix. What special considerations (e.g., mechanical testing, histological analysis, spatial distribution of gene expression, etc.) does this present for product characterization and specifications? Please discuss in terms of product safety, purity, identity, and potency.

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