V. 参考資料

１．ヒト（自己・同種）由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針
1-1. 平成 20 年 9 月 12 日付「平成 20 年 2 月 8 日付　ヒト（自己）由来細胞や組織を加工した医薬品又は医療機器の品質及び安全性の確保についての修正後の通知」
1-2. 平成 20 年 9 月 12 日付「ヒト（同種）由来細胞や組織を加工した医薬品又は医療機器の品質及び安全性の確保について」

２．Guidance for Industry Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage (U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research Center for Devices and Radiological Health, 2007)

３．Draft Reflection Paper on In-Vitro Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee (European Medicines Agency, EMEA/CAT/CPWP/288934/2009)

４．Assesssment Report for ChondroCelect and Annex I (European Medicines Agency, EMEA/724428/2009)

〔参　考〕修正後の通知

薬食発第0208003号
平成２０年２月８日

各都道府県知事 殿

厚生労働省医薬食品局長

ヒト（自己）由来細胞や組織を加工した医薬品又は医療機器の
品質及び安全性の確保について

ヒト由来の細胞・組織を加工した医薬品又は医療機器の品質及び安全性を確保するために必要な基本的要件については、平成12年12月26日付け医薬発第1314号厚生省医薬安全局長通知「ヒト又は動物由来成分を原料として製造される医薬品等の品質及び安全性確保について」の別添２「ヒト由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針」（以下「平成12年指針」という。）を定め運用してきたが、その後の科学技術の進歩や経験の蓄積を踏まえ見直しを進めてきたところである。

今般、ヒト由来の細胞・組織のうち、自己由来の細胞・組織を加工した医薬品又は医療機器（以下「自己由来細胞・組織加工医薬品等」という。）の品質及び安全性の確保のための基本的な技術要件について別添「ヒト（自己）由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針」のとおりとりまとめ、自己由来細胞・組織加工医薬品等については、平成12年指針に代え本指針によることとしたので、御了知の上、貴管下関係団体、関係機関等に周知願いたい。

なお、ヒト由来細胞・組織のうち、自己以外の同種由来の細胞・組織を加工した医薬品又は医療機器の品質及び安全性の確保のための基本的な技術要件についてもとりまとめているところであり、おって通知する予定であることを申し添える。

ヒト（自己）由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針

はじめに

１．　本指針は、ヒト由来細胞・組織のうち、自己由来細胞・組織を加工した医薬品又は医療機器（以下「細胞・組織加工医薬品等」という。）の品質及び安全性の確保のための基本的な技術要件について定めるものである。

しかしながら、細胞・組織加工医薬品等の種類や特性、臨床上の適用法は多種多様であり、また、本分野における科学的進歩や経験の蓄積は日進月歩である。本指針を一律に適用したり、本指針の内容が必要事項すべてを包含しているとみなすことが必ずしも適切でない場合もある。したがって、個々の医薬品等についての試験の実施や評価に際しては本指針の目的を踏まえ、その時点の学問の進歩を反映した合理的根拠に基づき、ケース・バイ・ケースで柔軟に対応することが必要であること。

２．　平成11年7月30日付け医薬発第906号厚生省医薬安全局長通知「細胞・組織を利用した医療用具又は医薬品の品質及び安全性の確保について」による確認申請時点における本指針への適合性の確認の趣旨は、当該細胞・組織加工医薬品等の治験を開始するに当たって支障となる品質及び安全性上の問題が存在するか否かの確認にある。したがって、確認申請の場合、その申請に当たって添付するべき資料について本指針に示された要件や内容をすべて充たすことを必ずしも求めている訳ではない。製造販売承認申請時における品質及び安全性の確保のための資料は治験の進行とともに本指針に沿って充実整備されることを前提に、確認申請では、当該時点でその趣旨に適う条件を充たし、合理的に作成された適切な資料を提出すること。

また、確認に必要とされる資料の範囲及び程度については、当該製品の由来、対象疾患、対象患者、適用部位、適用方法及び加工方法等により異なり、本指針では具体的に明らかでないことも少なくないので、個別に独立行政法人医薬品医療機器総合機構に相談することが望ましい。

目次

第１章　総則

第１　目的

本指針は、ヒト由来細胞・組織のうち、自己由来細胞・組織を加工した医薬品又は医療機器（以下「細胞・組織加工医薬品等」という。）の品質及び安全性の確保のための基本的な技術要件について定めるものである。

第２　定義

本指針における用語の定義は以下のとおりとする。

１　「細胞・組織の加工」とは、疾患の治療や組織の修復又は再建を目的として、細胞・組織の人為的な増殖、細胞・組織の活性化等を目的とした薬剤処理、生物学的特性改変、非細胞・組織成分との組み合わせ又は遺伝子工学的改変等を施すことをいう。

組織の分離、組織の細切、細胞の分離、特定細胞の単離、抗生物質による処理、洗浄、ガンマ線等による滅菌、冷凍、解凍等は加工とみなさない。

２　「製造」とは、加工に加え、組織の分離、組織の細切、細胞の分離、特定細胞の単離、抗生物質による処理、洗浄、ガンマ線等による滅菌、冷凍、解凍等、当該細胞・組織の本来の性質を改変しない操作を含む行為で、最終製品である細胞・組織利用製品を出荷するまでに行う行為をいう。

３　「表現型」とは、ある一定の環境条件のもとで、ある遺伝子によって表現される形態学的及び生理学的な性質をいう。

４　「ドナー」とは、細胞・組織加工医薬品等の原料となる細胞・組織を提供するヒトをいう。自己由来細胞・組織加工医薬品等にあっては、患者はドナーである。

５　「遺伝子導入構成体」とは、目的遺伝子を標的細胞に導入するための運搬体、目的遺伝子及びその機能発現に必要な要素をコードする塩基配列等から構成されるものをいう。

第２章　製造方法

第１　原材料及び製造関連物質

１　目的とする細胞・組織

(1)　生物学的構造・機能の特徴と選択理由

原材料として用いられる細胞・組織について、その生物学的構造・機能の特徴を、例えば、形態学的特徴、増殖特性、生化学的指標、免疫学的指標、特徴的産生物質その他適切な遺伝型又は表現型の指標から適宜選択して示し、当該細胞・組織を原料として選択した理由を説明すること。

(2)　ドナーの感染症に対する留意点

患者、製造従事者及び医療従事者の安全性を確保する観点等から、採取細胞・組織を介して感染する可能性がある各種感染症を考慮して感染症に関する検査項目を定め、その妥当性を明らかにすること。特にＢ型肝炎(HBV)、Ｃ型肝炎(HCV)、

ヒト免疫不全ウイルス（HIV）感染症、成人Ｔ細胞白血病（HTLV）に留意すること。

(3) 細胞・組織の採取・保存・運搬

① 採取者及び採取医療機関等の適格性

採取者及び採取医療機関等に求めるべき技術的要件について、明らかにすること。

② 採取部位及び採取方法の妥当性

細胞の採取部位の選定基準、採取方法を示し、これらが科学的及び倫理的に適切に選定されたものであることを明らかにすること。採取方法については、用いられる器具、微生物汚染防止、取り違えやクロスコンタミネーション防止のための方策等を具体的に示すこと。

③ ドナーに対する説明及び同意

細胞・組織採取時のドナーに対する説明及び同意の内容を規定すること。

④ ドナーの個人情報の保護

ドナーの個人情報の保護方策について具体的に規定すること。

⑤ ドナーの安全性確保のための試験検査

細胞・組織採取時にドナーの安全性確保のために採取部位の状態の確認など試験検査を行わなければならない場合には、その内容、検査結果等に問題があった場合の対処法について具体的に規定すること。

⑥ 保存方法及び取り違え防止策

採取した細胞・組織を一定期間保存する必要がある場合には、保存条件や保存期間及びその設定の妥当性について明らかにすること。また、取り違えを避けるための手段や手順等について具体的に規定すること。

⑦ 運搬方法

採取細胞・組織を運搬する必要がある場合には、運搬容器、運搬手順（温度管理等を含む。）を定め、その妥当性について明らかにすること。

⑧ 記録の作成及び保管方法

①～⑦に関する事項について、実施の記録を文書で作成し、適切に保管する方法について明らかにすること。

2　目的とする細胞・組織以外の原材料及び製造関連物質

目的とする細胞・組織以外の原材料及び製造関連物質を明らかにし、その適格性を示すとともに、必要に応じて規格を設定し、適切な品質管理を行うことが必要である。

なお、生物由来製品又は特定生物由来製品を原材料として使用する場合は、その使用量を必要最小限とし、「生物由来原料基準」（平成15年厚生労働省告示第210号）をはじめとする関連法令及び通知を遵守すること。特に、ウイルス不活化及び除去に関する情報を十分に評価する必要があるほか、遡及調査等を確保する方策についても明らかにすること。

(1) 細胞の培養を行う場合

① 培地、添加成分（血清、成長因子及び抗生物質等）及び細胞の処理に用いる試薬等のすべての成分等についてその適格性を明らかにし、必要に応じて規格を設定すること。各成分等の適格性の判定及び規格の設定に当たっては、最終製品の適用経路等を考慮すること。

② 培地成分については、以下の点に留意すること。

ア　培地に使用する成分及び水は、可能な範囲で医薬品又は医薬品原料に相当する基準で品質管理されている生物学的純度の高い品質のものを使用すること。

イ　培地に使用する成分は主成分のみでなく使用するすべての成分について明らかにし、選択理由及び必要に応じて品質管理法等を明確にすること。ただし、培地の構成成分が周知のもので、市販品等が一般的に使用されている DMEM、MCDB、HAM、RPMI のような培地は１つのものと考えてよい。

ウ　すべての成分を含有した培地の最終品については、無菌性及び目的とした培養に適していることを判定するための性能試験を実施する必要がある。その他、工程管理上必要と思われる試験項目を規格として設定し、適切な品質管理を行う必要がある。

③ 異種血清及び異種もしくは同種の血清に由来する成分については、細胞活性化又は増殖等の加工に必須でなければ使用しないこと。特に繰り返して使用する可能性のある製品では可能な限り使用を避けるよう検討すること。血清等の使用が避けられない場合には、以下の点を考慮し、血清等からの細菌、真菌、ウイルス及び異常プリオン等の混入・伝播を防止するとともに、最終製品から可能な限り除去するよう処理方法等を検討すること。

ア　血清等の由来を明確にすること。

イ　牛海綿状脳症発生地域からの血清を極力避ける等感染症リスクの低減に努めること。

ウ　由来動物種に特異的なウイルスやマイコプラズマに関する適切な否定試験を行い、ウイルス等に汚染されていないことを確認した上で使用すること。

エ　細胞の活性化、増殖に影響を与えない範囲で細菌、真菌及びウイルス等に対する適切な不活化処理及び除去処理を行う。例えば、潜在的なウイルス混入の危険性を避けるために、必要に応じて加熱処理、フィルター処理、放射線処理又は紫外線処理等を組み合わせて行うこと。

オ　培養細胞でのウイルス感染のモニター、患者レベルでのウイルス性疾患の発症に対するモニター及び異種血清成分に対する抗体産生等の調査のために、使用した血清の一部を保管すること。

④ 抗生物質の使用は極力避けるべきである。ただし製造初期の工程において抗生物質の使用が不可欠と考えられる場合には、その後の工程で可能な限り漸減を図るほか、その科学的理由、最終製品での推定残存量、患者に及ぼす影響などの面から妥当性を説明すること。また、用いる抗生物質に過敏症の既往歴のある患者の場合には、本治療を適応すべきではない。なお、抗生物質を使用する場合でも十分に除去されることが立証される場合には、その使用を妨げるものではない。

⑤ 成長因子を用いる場合には、細胞培養特性の再現性を保証するために、例えば純度及び力価に関する規格を設定する等適切な品質管理法を示すこと。
⑥ 最終製品に含有している可能性のある培地成分や操作のために用いられたその他の成分等については、生体に悪影響を及ぼさないものを選択すること。
⑦ フィーダー細胞として異種動物由来の細胞を用いる場合には、異種動物由来の感染症のリスクの観点から安全性を確保すること。

(2) 非細胞・組織成分と組み合わせる場合

① 細胞・組織以外の原材料の品質及び安全性について

細胞・組織とともに最終製品の一部を構成する細胞・組織以外の原材料（マトリックス、医療材料、スキャフォールド、支持膜、ファイバー及びビーズ等）がある場合には、その品質及び安全性に関する知見について明らかにすること。

当該原材料の種類と特性、最終製品における形態・機能及び想定される臨床適応の観点から見た品質、安全性及び有効性評価との関連を勘案して、適切な情報を提供すること。生体吸収性材料を用いる場合には、分解生成物に関して必要な試験を実施すること。

なお、必要な試験等については、平成15年2月13日付け医薬審発第0213001号厚生労働省医薬食品局審査管理課長通知「医療用具の製造（輸入）承認申請に必要な生物学的試験の基本的考え方について」等を参照し、試験結果及び当該原材料を使用することの妥当性を示すこと。文献からの知見、情報を合理的に活用すること。

② 目的とする細胞・組織との相互作用について

細胞・組織との相互作用に関し、以下の事項について、確認方法及び確認結果を示すこと。

ア　非細胞・組織成分が、想定される臨床適応に必要な細胞・組織の機能、生育能力、活性及び安定性に悪影響を与えないこと。

イ　非細胞・組織成分との相互作用によって起こり得る、細胞の変異、形質転換及び脱分化等を考慮し、その影響を可能な範囲で評価すること。

ウ　細胞との相互作用によって、想定される臨床適応において非細胞・組織成分に期待される性質が損なわれないこと。

(3) 細胞に遺伝子工学的改変を加える場合

細胞に遺伝子を導入する場合は、次に掲げる事項に関する詳細を示すこと。

① 目的遺伝子の構造、由来、入手方法、クローニング方法並びにセル・バンクの調製方法、管理方法及び更新方法等に関する情報
② 導入遺伝子の性質
③ 目的遺伝子産物の構造、生物活性及び性質
④ 遺伝子導入構成体を作製するために必要なすべての原材料、性質及び手順（遺伝子導入法並びに遺伝子導入用ベクターの由来、性質及び入手方法等）
⑤ 遺伝子導入構成体の構造や特性

⑥ ベクターや遺伝子導入構成体を作製するための細胞やウイルスのバンク化及びバンクの管理方法

遺伝子導入細胞の製造方法については、平成７年11月15日付け薬発第1062号厚生省薬務局長通知「遺伝子治療用医薬品の品質及び安全性の確保に関する指針について」（以下、「遺伝子治療用医薬品指針」という。）の別添「遺伝子治療用医薬品の品質及び安全性の確保に関する指針」第２章等を参照すること。また、同通知の別記に準じて設定の妥当性等を明らかにすること。

なお、遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律（平成15年法律第97号）に基づき、「ヒトの細胞等」若しくは「分化する能力を有する、又は分化した細胞等であって、自然条件において個体に成育しないもの」以外の細胞、「ウイルス」及び「ウイロイド」に対して遺伝子工学的改変を加える場合には、別途手続きが必要となるので留意すること。

第２　製造工程

細胞・組織加工医薬品等の製造に当たっては、製造方法を明確にし、可能な範囲でその妥当性を以下の項目で検証し、品質の一定性を保持すること。

１　ロット構成の有無とロットの規定

製品がロットを構成するか否かを明らかにすること。ロットを構成する場合には、ロットの内容について規定しておくこと。

２　製造方法

原材料となる細胞・組織の受け入れから最終製品に至る製造の方法の概要を示すとともに、具体的な処理内容及び必要な工程管理、品質管理の内容を明らかにすること。

(1)　受入検査

採取した細胞・組織について、細胞・組織の種類や使用目的に応じて実施する受入のための試験検査の項目（例えば、目視検査、顕微鏡検査、採取収率、生存率、細胞・組織の特性解析及び微生物試験等）と各項目の判定基準を設定すること。確認申請段階にあっては、それまでに得られた試験検体での実測値を提示し、これらを踏まえた暫定値を示すこと。

(2)　細菌、真菌及びウイルス等の不活化・除去

採取した細胞・組織について、その細胞生存率や表現型、遺伝形質及び特有の機能その他の特性及び品質に影響を及ぼさない範囲で、必要かつ可能な場合は細菌、真菌及びウイルス等を不活化又は除去する処理を行うこと。当該処理に関する方策と評価方法について明らかにすること。

(3)　組織の細切、細胞の分離、特定細胞の単離等

採取した細胞・組織から製品を製造する初期の過程で行われる組織の細切、細胞の分離、特定細胞の単離及びそれらの洗浄等の方法を明らかにすること。特定細胞の単離を行う場合には、その確認方法を設定すること。

(4) 培養工程

製造工程中に培養工程が含まれる場合は、培地、培養条件、培養期間及び収率等を明らかにすること。

(5) 細胞のバンク化

細胞・組織加工医薬品等の製造のいずれかの過程で、細胞をバンク化する場合には、その理由、セル・バンクの作製方法及びセル・バンクの特性解析、保存・維持・管理方法・更新方法その他の各作業工程や試験に関する手順等について詳細を明らかにし、妥当性を示すこと。平成12年7月14日付け医薬審第873号厚生省医薬安全局審査管理課長通知「生物薬品（バイオテクノロジー応用医薬品／生物起源由来医薬品）製造用細胞基剤の由来、調製及び特性解析について」等を参考とすること。

(6) 製造工程中の取り違え及びクロスコンタミネーション防止対策

細胞・組織加工医薬品等の製造にあたっては、製造工程中の取り違え及びクロスコンタミネーションの防止が重要であり、工程管理における防止対策を明らかにすること。

3 加工した細胞の特性解析

加工した細胞について、加工に伴う変化を調べるために、例えば、形態学的特徴、増殖特性、生化学的指標、免疫学的指標、特徴的産生物質、その他適切な遺伝型又は表現型の指標を解析するとともに、必要に応じて機能解析を行うこと。

また、培養期間の妥当性及び細胞の安定性を評価するために、予定の培養期間を超えて培養した細胞において目的外の変化がないことを示すこと。

4 最終製品の形態、包装

最終製品の形態、包装は、製品の品質を確保できるものでなければならない。

5 製造方法の恒常性

細胞・組織加工医薬品等の製造に当たっては、製造工程を通じて、個別に加工した製品の細胞数、細胞生存率並びに製品の使用目的及び適用方法等からみた特徴（表現型の適切な指標、遺伝型の適切な指標、機能特性及び目的とする細胞の含有率等）が製品（ロット）間で本質的に損なわれないことを、試験的検体を用いてあらかじめ評価しておくこと。

製造工程中の凍結保存期間や加工に伴う細胞培養の期間が長期に及ぶ場合には一定期間ごとに無菌試験を行うなど、無菌性が確保されることを確認すること。

6 製造方法の変更

開発途中に製造方法を変更した場合、変更前の製造方法による製品を用いて得た試験成績を確認申請又は承認申請に使用するときは、製造方法変更前後の製品の同等性及び同質性を示すこと。

第３　最終製品の品質管理

１　総論

細胞・組織加工医薬品等の品質管理全体の方策としては、最終製品の規格及び試験方法の設定、個別患者への適用ごとの原材料の品質管理、製造工程の妥当性の検証と一定性の維持管理のほか、中間製品の品質管理を適正に行うこと等が挙げられる。

最終製品の規格及び試験方法については、対象とする細胞・組織の種類及び性質、製造方法、各製品の使用目的や使用方法、安定性、利用可能な試験法等によって異なると考えられるため、取り扱う細胞・組織によってこれらの違いを十分に考慮して設定すること。また、製造工程の妥当性の検証と一定性の維持管理法、中間製品の品質管理等との相互補完関係を考慮に入れて、全体として品質管理の目的が達成されるとの観点から、合理的に規格及び試験方法を設定し、その根拠を示すこと。なお、確認申請は、治験を実施する製品の品質として問題がないとみなせることを確認することを目的としている。したがって、無菌性やマイコプラズマの否定など必須なものを除き、治験後に臨床試験成績と品質の関係を論ずるために必要な品質特性については、やむを得ない場合は少数の試験的検体の実測値をもとにその変動をしかるべき範囲内に設定する暫定的な規格及び試験方法を設定することで差し支えない。ただし、規格及び試験方法を含む品質管理法は治験の進行とともに充実・整備を図ること。

２　最終製品の品質管理法

最終製品について、以下に示す一般的な品質管理項目及び試験を参考として、必要で適切な規格及び試験方法を設定し、その根拠を明らかにすること。

ロットを構成しない製品を製造する場合は個別製品ごとに、ロットを構成する製品を製造する場合には、通常、各個別製品ではなく各ロットが品質管理の対象となるので、これを踏まえてそれぞれ適切な規格、試験方法を設定すること。

(1)　細胞数並びに生存率

得られた細胞の数と生存率は、最終製品又は必要に応じて適切な製造工程の製品で測定すること。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(2)　確認試験

目的とする細胞・組織の形態学的特徴、生化学的指標、免疫学的指標、特徴的産生物質その他適切な遺伝型あるいは表現型の指標を選択して、目的とする細胞・組織であることを確認すること。

(3)　細胞の純度試験

目的細胞以外の異常増殖細胞、形質転換細胞の有無や混入細胞の有無等の細胞の純度について、目的とする細胞・組織の由来、培養条件等の製造工程等を勘案し、必要に応じて試験項目、試験方法及び判定基準を示すこと。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定する

ことでも良い。

(4) 細胞由来の目的外生理活性物質に関する試験

細胞由来の各種目的外生理活性物質のうち、製品中での存在量如何で患者に安全性上の重大な影響を及ぼす可能性が明らかに想定される場合には、適切な許容量限度試験を設定すること。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(5) 製造工程由来不純物試験

原材料に存在するか又は製造過程で非細胞・組織成分、培地成分、資材、試薬等に由来し、製品中に混入物、残留物、又は新たな生成物、分解物等として存在する可能性があるもので、かつ、品質及び安全性の面からみて望ましくない物質等（例えば、ウシ胎児血清由来のアルブミン、抗生物質等）については、当該物質の除去に関するプロセス評価や当該物質に対する工程内管理試験の結果を考慮してその存在を否定するか、又は適切な試験を設定して存在許容量を規定すること。試験対象物質の選定及び規格値の設定に当たっては、設定の妥当性について明らかにすること。

なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(6) 無菌試験及びマイコプラズマ否定試験

最終製品の無菌性については、あらかじめモデル検体を用いて全製造工程を通じて無菌性を確保できることを十分に評価しておく必要がある。最終製品について、患者に適用する前に無菌性（一般細菌及び真菌否定）を試験により示すこと。また、適切なマイコプラズマ否定試験を実施すること。最終製品の無菌試験等の結果が、患者への投与後にしか得られない場合には、投与後に無菌性等が否定された場合の対処方法をあらかじめ設定しておくこと。また、この場合、中間製品で無菌性を試験により示し、最終製品に至る工程の無菌性を厳密に管理する必要がある。また、同一施設・同一工程で以前に他の患者への適用例がある場合には、全例において試験により無菌性が確認されていること。ロットを構成する製品で密封性が保証されている場合には、代表例による試験でよい。適用ごとに試験を実施する必要がある場合で、無菌試験等の結果が、患者への投与後にしか得られない場合には、適用の可否は直近のデータを参考にすることになるが、この場合でも最終製品の無菌試験等は必ず行うこと。

抗生物質は細胞培養系で極力使用しないことが望まれるが、使用した場合には、無菌試験に影響を及ぼさないよう処置すること。

(7) エンドトキシン試験

試料中の夾雑物の影響を考慮して試験を実施すること。規格値は必ずしも実測値によらず、日本薬局方等で示されている最終製品の１回投与量を基にした安全域を考慮して設定すればよい。また、工程内管理試験として設定することも考えられるが、その場合には、バリデーションの結果を含めて基準等を設定し、その妥当性を説明すること。

(8) ウイルス試験

HBV、HCV、HIV、HTLVを増殖させる可能性のある細胞の場合には、中間製品、最終製品等について、増殖可能性のあるウイルスについてその存在量に関する試験を実施し、細胞・組織加工医薬品等の投与が患者の不利益にならないことを確認する必要がある。また、製造工程中で生物由来成分を使用する場合には、最終製品で当該成分由来のウイルスについての否定試験の実施を考慮すべき場合もあるかも知れない。しかし可能な限り、もとの成分段階での試験やプロセス評価で迷入が否定されていることが望ましい。

(9) 効能試験

幹細胞、リンパ球、遺伝子改変細胞その他の細胞等、臨床使用目的又は特性に応じた適切な効能試験の実施を考慮すべき場合もある。なお、確認申請においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

(10) 力価試験

細胞・組織から分泌される特定の生理活性物質の分泌が当該細胞・組織加工医薬品等の効能又は効果の本質である場合には、その目的としている必要な効果を発揮することを示すために、当該生理活性物質に関する検査項目及び規格を設定すること。遺伝子を導入した場合の発現産物又は細胞から分泌される目的の生成物等について、力価、産生量等の規格を設定すること。なお、確認申請時においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

(11) 力学的適合性試験

一定の力学的強度を必要とする製品については、適用部位を考慮した力学的適合性及び耐久性を確認するための規格を設定すること。なお、確認申請時においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

第３章　細胞・組織加工医薬品等の安定性

製品化した細胞・組織加工医薬品等又は重要なそれらの中間製品について、保存・流通期間及び保存形態を十分考慮して、細胞の生存率及び力価等に基づく適切な安定性試験を実施し、貯法及び有効期限を設定し、その妥当性を明らかにすること。特に凍結保管及び解凍を行う場合には、凍結及び解凍操作による製品の安定性や規格への影響がないかを確認すること。また、必要に応じて標準的な製造期間を超える場合や標準的な保存期間を超える長期保存についても検討し、安定性の限界を可能な範囲で確認すること。ただし、製品化後直ちに使用するような場合はこの限りではない。

また、製品化した細胞・組織加工医薬品等を運搬する場合には、運搬容器及び運搬手順（温度管理等を含む）等を定め、その妥当性について明らかにすること。

第４章　細胞・組織加工医薬品等の非臨床安全性試験

製品の特性及び適用法から評価が必要と考えられる安全性関連事項について、技術的に可能であれば、科学的合理性のある範囲で、適切な動物を用いた試験又は*in vitro* で

の試験を実施すること。なお、非細胞・組織成分及び製造工程由来の不純物等については、可能な限り、動物を用いた試験ではなく理化学的分析法により評価すること。

ヒト由来の試験用検体は貴重であり、また、ヒト由来の製品を実験動物等で試験して必ずしも意義ある結果が得られるとは限らない。このため、動物由来の製品モデルを作成し適切な実験動物に適用する試験系により試験を行うことで、より有用な知見が得られると考えられる場合には、むしろ、このような試験系を用いることに科学的合理性がある場合がある。場合によっては細胞を用いる試験系も考慮し、このようなアプローチにより試験を行った際には、その試験系の妥当性について明らかにすること。

以下に、必要に応じて非臨床的に安全性を確認する際の参考にすべき事項及び留意点の例を示す。これらは例示であって、合理性のない試験の実施を求める趣旨ではなく、製品の特性等を考慮して適切な試験を検討すること。

1　培養期間を超えて培養した細胞について、目的外の形質転換を起こしていないことを明らかにすること。

2　必要に応じて細胞・組織が産生する各種サイトカイン、成長因子等の生理活性物質の定量を行い、生体内へ適用したときの影響に関して考察を行うこと。

3　製品の適用が患者等の正常な細胞又は組織に影響を与える可能性について検討、考察すること。

4　製品及び導入遺伝子の発現産物等による望ましくない免疫反応が生じる可能性について検討、考察すること。

5　製造工程で外来遺伝子の導入が行われている場合には、遺伝子治療用医薬品指針に定めるところに準じて試験を行うこと。特に、ウイルスベクターを使用した場合には増殖性ウイルスがどの程度存在するかを検査するとともに、検査方法が適切であることについても明らかにすること。

また、導入遺伝子及びその産物の性状について調査し、安全性について明らかにすること。細胞については、増殖性の変化、腫瘍形成及びがん化の可能性について考察し、明らかにすること。

6　動物由来のモデル製品を含めて製品の入手が容易であり、かつ臨床上の適用に関連する有用な安全性情報が得られる可能性がある場合には、合理的に設計された一般毒性試験の実施を考慮すること。

なお、一般毒性試験の実施に当たっては、平成元年9月11日付け薬審1第24号厚生省薬務局新医薬品課長・審査課長連名通知「医薬品の製造（輸入）承認申請に必要な毒性試験のガイドラインについて」の別添「医薬品毒性試験法ガイドライン」等を参照すること。

第５章　細胞・組織加工医薬品等の効力又は性能を裏付ける試験

1　技術的に可能かつ科学的に合理性のある範囲で、実験動物又は細胞等を用い、適切に設計された試験により、細胞・組織加工医薬品等の機能発現、作用持続性及び医薬品・医療機器として期待される効果を検討すること。

2　遺伝子導入細胞にあっては、導入遺伝子からの目的産物の発現効率及び発現の持続性、導入遺伝子の発現産物の生物活性並びに医薬品等として期待される効果等を

検討すること。

3　適当な動物由来細胞・組織製品モデル又は疾患モデル動物がある場合には、それを用いて治療効果を検討すること。

4　確認申請段階では、当該製品の効力又は性能による治療が他の治療法と比較したときはるかに優れて期待できることが国内外の文献又は知見等により合理的に明らかにされている場合には、必ずしも詳細な実験的検討は必要とされない。

第６章　細胞・組織加工医薬品等の体内動態

1　製品を構成する細胞・組織及び導入遺伝子の発現産物について、技術的に可能で、かつ、科学的合理性がある範囲で、実験動物での吸収及び分布等の体内動態に関する試験等により、患者等に適用された製品中の細胞・組織の生存期間、効果持続期間を推測し、目的とする効果が十分得られることを明らかにすること。

2　当該細胞・組織が特定の部位（組織等）に到達して作用する場合には、その局在性を明らかにすること。

第７章　臨床試験

確認申請の段階における安全性については、臨床上の有用性を勘案して評価されるものであり、細胞・組織加工医薬品等について予定されている国内の治験計画について以下の項目を踏まえて評価すること。

1　対象疾患

2　対象とする被験者及び被験者から除外すべき患者の考え方

3　細胞・組織加工医薬品等の適用を含め、被験者に対して行われる治療内容

4　既存の治療法との比較を踏まえた臨床試験実施の妥当性

5　現在得られている情報から想定されるリスク及びベネフィットを含め、被験者への説明事項の案

なお、臨床試験は、適切な試験デザイン及びエンドポイントを設定して実施する必要があり、目的とする細胞・組織の由来、対象疾患及び適用方法等を踏まえて適切に計画すること。

薬食発第0912006号
平成２０年９月１２日

各都道府県知事 殿

厚生労働省医薬食品局長

ヒト（同種）由来細胞や組織を加工した医薬品又は医療機器の
品質及び安全性の確保について

ヒト由来の細胞・組織を加工した医薬品又は医療機器（以下「細胞・組織加工医薬品等」という。）の品質及び安全性を確保するための基本的な技術要件については、平成12年12月26日付け医薬発第1314号厚生省医薬安全局長通知「ヒト又は動物由来成分を原料として製造される医薬品等の品質及び安全性確保について」の別添２「ヒト由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針」（以下「平成12年指針」という。）を定め運用してきたが、その後の科学技術の進歩や経験の蓄積を踏まえ見直しを進めてきたところである。

ヒトの自己由来の細胞・組織加工医薬品等の品質及び安全性の確保のための基本的な技術要件については、平成20年２月８日付け薬食発第0208003号厚生労働省医薬食品局長通知「ヒト（自己）由来細胞や組織を加工した医薬品又は医療機器の品質及び安全性の確保について」により通知したところであるが、今般、ヒトの同種由来の細胞・組織加工医薬品等の品質及び安全性の確保のための基本的な技術要件についても、別添「ヒト（同種）由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針」のとおりとりまとめたので、御了知の上、貴管下関係団体、関係機関等に周知願いたい。

なお、これに伴い、平成12年指針は廃止することとする。

ヒト（同種）由来細胞・組織加工医薬品等の品質及び安全性の確保に関する指針

はじめに

１．　本指針は、ヒト由来細胞・組織のうち、同種由来細胞・組織（自己由来細胞・組織を除く。）を加工した医薬品又は医療機器（以下「細胞・組織加工医薬品等」という。)の品質及び安全性の確保のための基本的な技術要件について定めるものである。

しかしながら、細胞・組織加工医薬品等の種類や特性、臨床上の適用法は多種多様であり、また、本分野における科学的進歩や経験の蓄積は日進月歩である。本指針を一律に適用したり、本指針の内容が必要事項すべてを包含しているとみなすことが必ずしも適切でない場合もある。したがって、個々の医薬品等についての試験の実施や評価に際しては本指針の目的を踏まえ、その時点の学問の進歩を反映した合理的根拠に基づき、ケース・バイ・ケースで柔軟に対応することが必要であること。

２．　平成11年7月30日付け医薬発第906号厚生省医薬安全局長通知「細胞・組織を利用した医療用具又は医薬品の品質及び安全性の確保について」による確認申請時点における本指針への適合性の確認の趣旨は、当該細胞・組織加工医薬品等の治験を開始するに当たって支障となる品質及び安全性上の問題が存在するか否かの確認にある。したがって、確認申請の場合、その申請に当たって添付するべき資料について本指針に示された要件や内容をすべて充たすことを必ずしも求めている訳ではない。製造販売承認申請時における品質及び安全性の確保のための資料は治験の進行とともに本指針に沿って充実整備されることを前提に、確認申請では、当該時点でその趣旨に適う条件を充たし、合理的に作成された適切な資料を提出すること。

また、確認に必要とされる資料の範囲及び程度については、当該製品の由来、対象疾患、対象患者、適用部位、適用方法及び加工方法等により異なり、本指針では具体的に明らかでないことも少なくないので、個別に独立行政法人医薬品医療機器総合機構に相談することが望ましい。

目次

第１章　総則

第１　目的

本指針は、ヒト由来細胞・組織のうち、同種由来細胞・組織（自己由来のものを除く。）を加工した医薬品又は医療機器（以下「細胞・組織加工医薬品等」という。）の品質及び安全性の確保のための基本的な技術要件について定めるものである。

第２　定義

本指針における用語の定義は以下のとおりとする。

１　「細胞・組織の加工」とは、疾患の治療や組織の修復又は再建を目的として、細胞・組織の人為的な増殖、細胞の株化、細胞・組織の活性化等を目的とした薬剤処理、生物学的特性改変、非細胞・組織成分との組み合わせ又は遺伝子工学的改変等を施すことをいう。

組織の分離、組織の細切、細胞の分離、特定細胞の単離、抗生物質による処理、洗浄、ガンマ線等による滅菌、冷凍、解凍等は加工とみなさない。

２　「製造」とは、加工に加え、組織の分離、組織の細切、細胞の分離、特定細胞の単離、抗生物質による処理、洗浄、ガンマ線等による滅菌、冷凍、解凍等、当該細胞・組織の本来の性質を改変しない操作を含む行為で、最終製品である細胞・組織利用製品を出荷するまでに行う行為をいう。

３　「表現型」とは、ある一定の環境条件のもとで、ある遺伝子によって表現される形態学的及び生理学的な性質をいう。

４　「HLAタイピング」とは、ヒトの主要組織適合性抗原型であるHLA（ヒト白血球抗原）のタイプを特定することをいう。

５　「ドナー」とは、細胞・組織加工医薬品等の原料となる細胞・組織を提供するヒトをいう。

６　「遺伝子導入構成体」とは、目的遺伝子を標的細胞に導入するための運搬体、目的遺伝子及びその機能発現に必要な要素をコードする塩基配列等から構成されるものをいう。

第２章　製造方法

第１　原材料及び製造関連物質

１　目的とする細胞・組織

(1) 起源及び由来、選択理由

原材料として用いられる細胞・組織の起源及び由来について説明し、当該細胞・組織を選択した理由を明らかにすること。

(2) 原材料となる細胞・組織の特性と適格性

① 生物学的構造・機能の特徴と選択理由

原材料として用いられる細胞・組織について、その生物学的構造・機能の特徴を、例えば、形態学的特徴、増殖特性、生化学的指標、免疫学的指標、特徴的産

生物質、HLAタイピング、その他適切な遺伝型又は表現型の指標から適宜選択して示し、当該細胞・組織を原料として選択した理由を説明すること。

② ドナーの選択基準、適格性

ドナーが倫理的に適切に選択されたことを示すこと。また、年齢、性別、民族学的特徴、病歴、健康状態、採取細胞・組織を介して感染する可能性がある各種感染症に関する検査項目、免疫適合性等を考慮して、選択基準、適格性基準を定め、その妥当性を明らかにすること。

特にＢ型肝炎(HBV)、Ｃ型肝炎(HCV)、ヒト免疫不全ウイルス（HIV）感染症、成人Ｔ細胞白血病(HTLV)、パルボウイルスＢ１９感染症については、問診及び検査（血清学的試験や核酸増幅法等）により否定すること。また、サイトメガロウイルス感染、EBウイルス感染及びウエストナイルウイルス感染については必要に応じて検査により否定すること。

この他、次に掲げるものについては既往歴、問診等の診断を行うとともに、輸血、移植医療を受けた経験の有無等からドナーとしての適格性を判断すること。

・梅毒トレポネーマ、クラミジア、淋菌、結核菌等の細菌による感染症
・敗血症及びその疑い
・悪性腫瘍
・重篤な代謝及び内分泌疾患
・膠原病及び血液疾患
・肝疾患
・伝達性海綿状脳症及びその疑い並びにその他の認知症

(3) ドナーに関する記録

原材料となる細胞・組織について、安全性確保上必要な情報が確認できるよう、ドナーに関する記録が整備、保管されていること。また、その具体的方策を示すこと。

(4) 細胞・組織の採取・保存・運搬

① 採取者及び採取医療機関等の適格性

採取者及び採取医療機関等に求めるべき技術的要件について、明らかにすること。

② 採取部位及び採取方法の妥当性

細胞の採取部位の選定基準、採取方法を示し、これらが科学的及び倫理的に適切に選択されたものであることを明らかにすること。採取方法については、用いられる器具、微生物汚染防止、取り違えやクロスコンタミネーション防止のための方策等を具体的に示すこと。

③ ドナーに対する説明及び同意

細胞・組織採取時のドナーに対する説明及び同意の内容を規定すること。

④ ドナーの個人情報の保護

ドナーの個人情報の保護方策について具体的に規定すること。

⑤ ドナーの安全性確保のための試験検査

細胞・組織採取時にドナーの安全性確保のために採取部位の状態の確認など試験検査を行わなければならない場合には、その内容、検査結果等に問題があった場合の対処法について具体的に規定すること。

⑥ 保存方法及び取り違え防止策

採取した細胞・組織を一定期間保存する必要がある場合には、保存条件や保存期間及びその設定の妥当性について明らかにすること。また、取り違えを避けるための手段や手順等について具体的に説明すること。

⑦ 運搬方法

採取細胞・組織を運搬する必要がある場合には、運搬容器、運搬手順（温度管理等を含む。）を定め、その妥当性について明らかにすること。

⑧ 記録の作成及び保管方法

①～⑦に関する事項について、実施の記録を文書で作成し、適切に保管する方法について明らかにすること。

２　目的とする細胞・組織以外の原材料及び製造関連物質

目的とする細胞・組織以外の原材料及び製造関連物質を明らかにし、その適格性を示すとともに、必要に応じて規格を設定し、適切な品質管理を行うことが必要である。

なお、生物由来製品又は特定生物由来製品を原材料として使用する場合は、その使用量を必要最小限とし、「生物由来原料基準」（平成15年厚生労働省告示第210号）をはじめとする関連法令及び通知を遵守すること。特に、ウイルス不活化及び除去に関する情報を十分に評価する必要があるほか、遡及調査等を確保する方策についても明らかにすること。

(1) 細胞の培養を行う場合

① 培地、添加成分（血清、成長因子及び抗生物質等）及び細胞の処理に用いる試薬等のすべての成分等についてその適格性を明らかにし、必要に応じて規格を設定すること。各成分等の適格性の判定及び規格の設定に当たっては、最終製品の適用経路等を考慮すること。

② 培地成分については、以下の点に留意すること。

ア　培地に使用する成分及び水は、可能な範囲で医薬品又は医薬品原料に相当する基準で品質管理されている生物学的純度の高い品質のものを使用すること。

イ　培地に使用する成分は主成分のみでなく使用するすべての成分について明らかにし、選択理由及び必要に応じて品質管理法等を明確にすること。ただし、培地の構成成分が周知のもので、市販品等が一般的に使用されている DMEM、MCDB、HAM、RPMI のような培地は１つのものと考えてよい。

ウ　すべての成分を含有した培地の最終品については、無菌性及び目的とした培養に適していることを判定するための性能試験を実施する必要がある。その他、工程管理上必要と思われる試験項目を規格として設定し、適切な品質管理を行

う必要がある。

③ 異種血清及び異種もしくは同種の血清に由来する成分については、細胞活性化又は増殖等の加工に必須でなければ使用しないこと。特に繰り返して使用する可能性のある製品では可能な限り使用を避けるよう検討すること。血清等の使用が避けられない場合には、以下の点を考慮し、血清等からの細菌、真菌、ウイルス及び異常プリオン等の混入・伝播を防止するとともに、最終製品から可能な限り除去するよう処理方法等を検討すること。

ア　血清等の由来を明確にすること。

イ　牛海綿状脳症発生地域からの血清を極力避ける等感染症リスクの低減に努めること。

ウ　由来動物種に特異的なウイルスやマイコプラズマに関する適切な否定試験を行い、ウイルス等に汚染されていないことを確認した上で使用すること。

エ　細胞の活性化、増殖に影響を与えない範囲で細菌、真菌及びウイルス等に対する適切な不活化処理及び除去処理を行う。例えば、潜在的なウイルス混入の危険性を避けるために、必要に応じて加熱処理、フィルター処理、放射線処理又は紫外線処理等を組み合わせて行うこと。

オ　培養細胞でのウイルス感染のモニター、患者レベルでのウイルス性疾患の発症に対するモニター及び異種血清成分に対する抗体産生等の調査のために、使用した血清の一部を保管すること。

④ 抗生物質の使用は極力避けるべきである。ただし製造初期の工程において抗生物質の使用が不可欠と考えられる場合には、その後の工程で可能な限り漸減を図るほか、その科学的理由、最終製品での推定残存量、患者に及ぼす影響などの面から妥当性を説明すること。また、用いる抗生物質に過敏症の既往歴のある患者の場合には、本治療を適応すべきではない。なお、抗生物質を使用する場合でも十分に除去されることが立証される場合には、その使用を妨げるものではない。

⑤ 成長因子を用いる場合には、細胞培養特性の再現性を保証するために、例えば純度及び力価に関する規格を設定する等適切な品質管理法を示すこと。

⑥ 最終製品に含有している可能性のある培地成分や操作のために用いられたその他の成分等については、生体に悪影響を及ぼさないものを選択すること。

⑦ フィーダー細胞として異種動物由来の細胞を用いる場合には、異種動物由来の感染症のリスクの観点から安全性を確保すること。

(2) 非細胞・組織成分と組み合わせる場合

① 細胞・組織以外の原材料の品質及び安全性について

細胞・組織とともに最終製品の一部を構成する細胞・組織以外の原材料（マトリックス、医療材料、スキャフォールド、支持膜、ファイバー及びビーズ等）がある場合には、その品質及び安全性に関する知見について明らかにすること。

当該原材料の種類と特性、最終製品における形態・機能及び想定される臨床適応の観点から見た品質、安全性及び有効性評価との関連を勘案して、適切な情報を提供すること。生体吸収性材料を用いる場合には、分解生成物に関して必要な

試験を実施すること。

なお、必要な試験等については、平成15年2月13日付け医薬審発第0213001号厚生労働省医薬食品局審査管理課長通知「医療用具の製造（輸入）承認申請に必要な生物学的試験の基本的考え方について」等を参照し、試験結果及び当該原材料を使用することの妥当性を示すこと。文献からの知見、情報を合理的に活用すること。

② 目的とする細胞・組織との相互作用について

細胞・組織との相互作用に関し、以下の事項について、確認方法及び確認結果を示すこと。

ア　非細胞・組織成分が、想定される臨床適応に必要な細胞・組織の機能、生育能力、活性及び安定性に悪影響を与えないこと。

イ　非細胞・組織成分との相互作用によって起こり得る、細胞の変異、形質転換及び脱分化等を考慮し、その影響を可能な範囲で評価すること。

ウ　細胞との相互作用によって、想定される臨床適応において非細胞・組織成分に期待される性質が損なわれないこと。

③ 細胞・組織と適用部位を隔離する目的で非細胞・組織成分を使用する場合

非細胞・組織成分を細胞・組織と適用部位を隔離する目的で使用する場合、下記の項目を参考に効果、安全性を確認すること。

ア　免疫隔離の程度

イ　細胞由来の目的生理活性物質の膜透過キネティクスと薬理効果

ウ　栄養成分及び排泄物の拡散

エ　非細胞・組織成分が適用部位周辺に及ぼす影響

(3) 細胞に遺伝子工学的改変を加える場合

細胞に遺伝子を導入する場合は、次に掲げる事項に関する詳細を示すこと。

① 目的遺伝子の構造、由来、入手方法、クローニング方法並びにセル・バンクの調製方法、管理方法及び更新方法等に関する情報

② 導入遺伝子の性質

③ 目的遺伝子産物の構造、生物活性及び性質

④ 遺伝子導入構成体を作製するために必要なすべての原材料、性質及び手順（遺伝子導入法並びに遺伝子導入用ベクターの由来、性質及び入手方法等）

⑤ 遺伝子導入構成体の構造や特性

⑥ ベクターや遺伝子導入構成体を作製するための細胞やウイルスのバンク化及びバンクの管理方法

遺伝子導入細胞の製造方法については、平成７年11月15日付け薬発第1062号厚生省薬務局長通知「遺伝子治療用医薬品の品質及び安全性の確保に関する指針について」（以下、「遺伝子治療用医薬品指針」という。）の別添「遺伝子治療用医薬品の品質及び安全性の確保に関する指針」第２章等を参照すること。また、同通知の別記に準じて設定の妥当性等を明らかにすること。

なお、遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する

法律(平成15年法律第97号）に基づき、「ヒトの細胞等」若しくは「分化する能力を有する、又は分化した細胞等であって、自然条件において個体に成育しないもの」以外の細胞、「ウイルス」及び「ウイロイド」に対して遺伝子工学的改変を加える場合には、別途手続きが必要となるので留意すること。

第２　製造工程

細胞・組織加工医薬品等の製造に当たっては、製造方法を明確にし、可能な範囲でその妥当性を以下の項目で検証し、品質の一定性を保持すること。

１　ロット構成の有無とロットの規定

製品がロットを構成するか否かを明らかにすること。ロットを構成する場合には、ロットの内容について規定しておくこと。

２　製造方法

原材料となる細胞・組織の受け入れから最終製品に至る製造の方法の概要を示すとともに、具体的な処理内容及び必要な工程管理、品質管理の内容を明らかにすること。

(1) 受入検査

原材料となる細胞・組織について、細胞・組織の種類や使用目的に応じて実施する受入のための試験検査の項目（例えば、目視検査、顕微鏡検査、採取収率、生存率、細胞・組織の特性解析及び微生物試験等）と各項目の判定基準を設定すること。確認申請段階にあっては、それまでに得られた試験検体での実測値を提示し、これらを踏まえた暫定値を示すこと。

(2) 細菌、真菌及びウイルス等の不活化・除去

原材料となる細胞・組織について、その細胞生存率や表現型、遺伝形質及び特有の機能その他の特性及び品質に影響を及ぼさない範囲で、必要かつ可能な場合は細菌、真菌及びウイルス等を不活化又は除去する処理を行うこと。当該処理に関する方策と評価方法について明らかにすること。

(3) 組織の細切、細胞の分離、特定細胞の単離等

原材料となる細胞・組織から製品を製造する初期の過程で行われる組織の細切、細胞の分離、特定細胞の単離及びそれらの洗浄等の方法を明らかにすること。特定細胞の単離を行う場合には、その確認方法を設定すること。

(4) 培養工程

製造工程中に培養工程が含まれる場合は、培地、培養条件、培養期間及び収率等を明らかにすること。

(5) 株化細胞の樹立と使用

株化細胞の樹立に当たっては、ドナーの遺伝的背景を理解したうえで樹立すること。樹立の方法を明確にし、可能な範囲でその妥当性を明らかにすること。

株化細胞の品質の均質性および安定性を保持するため、必要な特性解析要件（細胞純度、形態学的評価、表現型特異的マーカ、核型など）を同定してその基準を設定するとともに、安定性を維持したまま増殖が可能な継代数を示すこと。

株化細胞に関しては、適切な動物モデル等を利用し、腫瘍形成及びがん化の可能

性について考察し、明らかにすること。

(6) 細胞のバンク化

細胞・組織加工医薬品等の製造のいずれかの過程で、細胞をバンク化する場合には、その理由、セル・バンクの作製方法及びセル・バンクの特性解析、保存・維持・管理方法・更新方法その他の各作業工程や試験に関する手順等について詳細を明らかにし、妥当性を示すこと。平成12年7月14日付け医薬審第873号厚生省医薬安全局審査管理課長通知「生物薬品（バイオテクノロジー応用医薬品／生物起源由来医薬品）製造用細胞基剤の由来、調製及び特性解析について」等を参考とすること。

(7) 製造工程中の取り違え及びクロスコンタミネーション防止対策

細胞・組織加工医薬品等の製造にあたっては、製造工程中の取り違え及びクロスコンタミネーションの防止が重要であり、工程管理における防止対策を明らかにすること。

３　加工した細胞の特性解析

加工した細胞について、加工に伴う変化を調べるために、例えば、形態学的特徴、増殖特性、生化学的指標、免疫学的指標、特徴的産生物質、その他適切な遺伝型又は表現型の指標を解析するとともに、必要に応じて機能解析を行うこと。

また、培養期間の妥当性及び細胞の安定性を評価するために、予定の培養期間を超えて培養した細胞において目的外の変化がないことを示すこと。

４　最終製品の形態、包装

最終製品の形態、包装は、製品の品質を確保できるものでなければならない。

５　製造方法の恒常性

細胞・組織加工医薬品等の製造に当たっては、製造工程を通じて、個別に加工した製品の細胞数、細胞生存率並びに製品の使用目的及び適用方法等からみた特徴(表現型の適切な指標、遺伝型の適切な指標、機能特性及び目的とする細胞の含有率等)が製品（ロット）間で本質的に損なわれないことを、試験的検体を用いてあらかじめ評価しておくこと。

製造工程中の凍結保存期間や加工に伴う細胞培養の期間が長期に及ぶ場合には一定期間ごとに無菌試験を行うなど、無菌性が確保されることを確認すること。

６　製造方法の変更

開発途中に製造方法を変更した場合、変更前の製造方法による製品を用いて得た試験成績を確認申請又は承認申請に使用するときは、製造方法変更前後の製品の同等性及び同質性を示すこと。

第３　最終製品の品質管理

１　総論

細胞・組織加工医薬品等の品質管理全体の方策としては、最終製品の規格及び試

験方法の設定、個別患者への適用ごとの原材料の品質管理、製造工程の妥当性の検証と一定性の維持管理のほか、中間製品の品質管理を適正に行うこと等が挙げられる。

最終製品の規格及び試験方法については､対象とする細胞・組織の種類及び性質、製造方法、各製品の使用目的や使用方法、安定性、利用可能な試験法等によって異なると考えられるため、取り扱う細胞・組織によってこれらの違いを十分に考慮して設定すること。また、製造工程の妥当性の検証と一定性の維持管理法、中間製品の品質管理等との相互補完関係を考慮に入れて、全体として品質管理の目的が達成されるとの観点から、合理的に規格及び試験方法を設定し、その根拠を示すこと。なお、確認申請は、治験を実施する製品の品質として問題がないとみなせることを確認することを目的としている。したがって、無菌性やマイコプラズマの否定など必須なものを除き、治験後に臨床試験成績と品質の関係を論ずるために必要な品質特性については、やむを得ない場合は少数の試験的検体の実測値をもとにその変動をしかるべき範囲内に設定する暫定的な規格及び試験方法を設定することで差し支えない。ただし、規格及び試験方法を含む品質管理法は治験の進行とともに充実・整備を図ること。

２　最終製品の品質管理法

最終製品について、以下に示す一般的な品質管理項目及び試験を参考として、必要で適切な規格及び試験方法を設定し、その根拠を明らかにすること。

ロットを構成しない製品を製造する場合は個別製品ごとに、ロットを構成する製品を製造する場合には、通常、各個別製品ではなく各ロットが品質管理の対象となるので、これを踏まえてそれぞれ適切な規格、試験方法を設定すること。

(1) 細胞数並びに生存率

得られた細胞の数と生存率は、最終製品又は必要に応じて適切な製造工程の製品で測定すること。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(2) 確認試験

目的とする細胞・組織の形態学的特徴、生化学的指標、免疫学的指標、特徴的産生物質その他適切な遺伝型あるいは表現型の指標を選択して、目的とする細胞・組織であることを確認すること。

(3) 細胞の純度試験

目的細胞以外の異常増殖細胞、形質転換細胞の有無や混入細胞の有無等の細胞の純度について、目的とする細胞・組織の由来、培養条件等の製造工程等を勘案し、必要に応じて試験項目、試験方法及び判定基準を示すこと。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(4) 細胞由来の目的外生理活性物質に関する試験

細胞由来の各種目的外生理活性物質のうち、製品中での存在量如何で患者に安全性上の重大な影響を及ぼす可能性が明らかに想定される場合には、適切な許容量限

度試験を設定すること。なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(5) 製造工程由来不純物試験

原材料に存在するか又は製造過程で非細胞・組織成分、培地成分、資材、試薬等に由来し、製品中に混入物、残留物、又は新たな生成物、分解物等として存在する可能性があるもので、かつ、品質及び安全性の面からみて望ましくない物質等（例えば、ウシ胎児血清由来のアルブミン、抗生物質等）については、当該物質の除去に関するプロセス評価や当該物質に対する工程内管理試験の結果を考慮してその存在を否定するか、又は適切な試験を設定して存在許容量を規定すること。試験対象物質の選定及び規格値の設定に当たっては、設定の妥当性について明らかにすること。

なお、確認申請時においては、少数の試験的検体での実測値を踏まえた暫定的な規格を設定することでも良い。

(6) 無菌試験及びマイコプラズマ否定試験

最終製品の無菌性については、あらかじめモデル検体を用いて全製造工程を通じて無菌性を確保できることを十分に評価しておく必要がある。最終製品について、患者に適用する前に無菌性（一般細菌及び真菌否定）を試験により示すこと。また、適切なマイコプラズマ否定試験を実施すること。最終製品の無菌試験等の結果が、患者への投与後にしか得られない場合には、投与後に無菌性等が否定された場合の対処方法をあらかじめ設定しておくこと。また、この場合、中間製品で無菌性を試験により示し、最終製品に至る工程の無菌性を厳密に管理する必要がある。また、同一施設・同一工程で以前に他の患者への適用例がある場合には、全例において試験により無菌性が確認されていること。ロットを構成する製品で密封性が保証されている場合には、代表例による試験でよい。適用ごとに試験を実施する必要がある場合で、無菌試験等の結果が、患者への投与後にしか得られない場合には、適用の可否は直近のデータを参考にすることになるが、この場合でも最終製品の無菌試験等は必ず行うこと。

抗生物質は細胞培養系で極力使用しないことが望まれるが、使用した場合には、無菌試験に影響を及ぼさないよう処置すること。

(7) エンドトキシン試験

試料中の夾雑物の影響を考慮して試験を実施すること。規格値は必ずしも実測値によらず、日本薬局方等で示されている最終製品の１回投与量を基にした安全域を考慮して設定すればよい。また、工程内管理試験として設定することも考えられるが、その場合には、バリデーションの結果を含めて基準等を設定し、その妥当性を説明すること。

(8) ウイルス等の試験

バンク化されておらず、ウインドウピリオドが否定できず、HBV、HCV、HIV等を製造工程中に増殖させる可能性のある細胞を用いる際には、中間製品、最終製品等についてもウイルス等の存在を否定する適切な試験を実施すること。また、製造工程中で生物由来成分を使用する場合には、最終製品で当該成分由来のウイルスにつ

いての否定試験の実施を考慮すべき場合もあるかも知れない。しかし可能な限り、もとの成分段階での試験やプロセス評価で迷入が否定されていることが望ましい。

(9) 効能試験

幹細胞、リンパ球、遺伝子改変細胞その他の細胞等、臨床使用目的又は特性に応じた適切な効能試験の実施を考慮すべき場合もある。なお、確認申請においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

(10) 力価試験

細胞・組織から分泌される特定の生理活性物質の分泌が当該細胞・組織加工医薬品等の効能又は効果の本質である場合には、その目的としている必要な効果を発揮することを示すために、当該生理活性物質に関する検査項目及び規格を設定すること。遺伝子を導入した場合の発現産物又は細胞から分泌される目的の生成物等について、力価、産生量等の規格を設定すること。なお、確認申請時においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

(11) 力学的適合性試験

一定の力学的強度を必要とする製品については、適用部位を考慮した力学的適合性及び耐久性を確認するための規格を設定すること。なお、確認申請時においては、少数の試験的検体による実測値を踏まえた暫定的な規格を設定することでも良い。

第３章　細胞・組織加工医薬品等の安定性

製品化した細胞・組織加工医薬品等又は重要なそれらの中間製品について、保存・流通期間及び保存形態を十分考慮して、細胞の生存率及び力価等に基づく適切な安定性試験を実施し、貯法及び有効期限を設定し、その妥当性を明らかにすること。特に凍結保管及び解凍を行う場合には、凍結及び解凍操作による製品の安定性や規格への影響がないかを確認すること。また、必要に応じて標準的な製造期間を超える場合や標準的な保存期間を超える長期保存についても検討し、安定性の限界を可能な範囲で確認すること。ただし、製品化後直ちに使用するような場合はこの限りではない。

また、製品化した細胞・組織加工医薬品等を運搬する場合には、運搬容器及び運搬手順（温度管理等を含む）等を定め、その妥当性について明らかにすること。

第４章　細胞・組織加工医薬品等の非臨床安全性試験

製品の特性及び適用法から評価が必要と考えられる安全性関連事項について、技術的に可能であれば、科学的合理性のある範囲で、適切な動物を用いた試験又は*in vitro*での試験を実施すること。なお、非細胞・組織成分及び製造工程由来の不純物等については、可能な限り、動物を用いた試験ではなく理化学的分析法により評価すること。

ヒト由来の試験用検体は貴重であり、また、ヒト由来の製品を実験動物等で試験して必ずしも意義ある結果が得られるとは限らない。このため、動物由来の製品モデルを作成し適切な実験動物に適用する試験系により試験を行うことで、より有用な知見が得られると考えられる場合には、むしろ、このような試験系を用いることに科学的合理性がある場合がある。場合によっては細胞を用いる試験系も考慮し、このようなアプローチにより試験を行なった際には、その試験系の妥当性について明らかにする

こと。

以下に、必要に応じて非臨床的に安全性を確認する際の参考にすべき事項及び留意点の例を示す。これらは例示であって、合理性のない試験の実施を求める趣旨ではなく、製品の特性等を考慮して適切な試験を検討すること。

1　培養期間を超えて培養した細胞について、目的外の形質転換を起こしていないことを明らかにすること。

2　必要に応じて細胞・組織が産生する各種サイトカイン、成長因子等の生理活性物質の定量を行い、生体内へ適用したときの影響に関して考察を行うこと。

3　製品の適用が患者等の正常な細胞又は組織に影響を与える可能性について検討、考察すること。

4　製品及び導入遺伝子の発現産物等による望ましくない免疫反応が生じる可能性について検討、考察すること。

5　株化細胞を用いた場合には、適切な動物モデル等を利用し、腫瘍形成及びがん化の可能性について考察し、明らかにすること。

6　製造工程で外来遺伝子の導入が行われている場合には、遺伝子治療用医薬品指針に定めるところに準じて試験を行うこと。特に、ウイルスベクターを使用した場合には増殖性ウイルスがどの程度存在するかを検査するとともに、検査方法が適切であることについても明らかにすること。

また、導入遺伝子及びその産物の性状について調査し、安全性について明らかにすること。細胞については、増殖性の変化、腫瘍形成及びがん化の可能性について考察し、明らかにすること。

7　動物由来のモデル製品を含めて製品の入手が容易であり、かつ臨床上の適用に関連する有用な安全性情報が得られる可能性がある場合には、合理的に設計された一般毒性試験の実施を考慮すること。

なお、一般毒性試験の実施に当たっては、平成元年9月11日付け薬審1第24号厚生省薬務局新医薬品課長・審査課長連名通知「医薬品の製造（輸入）承認申請に必要な毒性試験のガイドラインについて」の別添「医薬品毒性試験法ガイドライン」等を参照すること。

第５章　細胞・組織加工医薬品等の効力又は性能を裏付ける試験

1　技術的に可能かつ科学的に合理性のある範囲で、実験動物又は細胞等を用い、適切に設計された試験により、細胞・組織加工医薬品等の機能発現、作用持続性及び医薬品・医療機器として期待される効果を検討すること。

2　遺伝子導入細胞にあっては、導入遺伝子からの目的産物の発現効率及び発現の持続性、導入遺伝子の発現産物の生物活性並びに医薬品等として期待される効果等を検討すること。

3　適当な動物由来細胞・組織製品モデル又は疾患モデル動物がある場合には、それを用いて治療効果を検討すること。

4　確認申請段階では、当該製品の効力又は性能による治療が他の治療法と比較したときはるかに優れて期待できることが国内外の文献又は知見等により合理的に明ら

かにされている場合には、必ずしも詳細な実験的検討は必要とされない。

第６章　細胞・組織加工医薬品等の体内動態

１　製品を構成する細胞・組織及び導入遺伝子の発現産物について、技術的に可能で、かつ、科学的合理性がある範囲で、実験動物での吸収及び分布等の体内動態に関する試験等により、患者等に適用された製品中の細胞・組織の生存期間、効果持続期間を推測し、目的とする効果が十分得られることを明らかにすること。

２　当該細胞・組織が特定の部位（組織等）に到達して作用する場合には、その局在性を明らかにすること。

第７章　臨床試験

確認申請の段階における安全性については、臨床上の有用性を勘案して評価されるものであり、細胞・組織加工医薬品等について予定されている国内の治験計画について以下の項目を踏まえて評価すること。

１　対象疾患

２　対象とする被験者及び被験者から除外すべき患者の考え方

３　細胞・組織加工医薬品等の適用を含め、被験者に対して行われる治療内容

４　既存の治療法との比較を踏まえた臨床試験実施の妥当性

５　現在得られている情報から想定されるリスク及びベネフィットを含め、被験者への説明事項の案

なお、臨床試験は、適切な試験デザイン及びエンドポイントを設定して実施する必要があり、目的とする細胞・組織の由来、対象疾患及び適用方法等を踏まえて適切に計画すること。

Guidance for Industry

Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage

DRAFT GUIDANCE

This guidance document is for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.fda.gov/dockets/ecomments. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions on the content of this guidance, contact Dr. Richard McFarland, Office of Cellular, Tissue and Gene Therapies, Center for Biologics Evaluation and Research, at 301-827-5102 or Mr. Aric Kaiser, Office of Device Evaluation, Center for Devices and Radiological Health, at 240-276-3676.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Center for Devices and Radiological Health
July 2007

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage

Additional copies of this guidance are available from:
Office of Communication, Training and Manufacturers Assistance, HFM-40
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448
Phone: 800-835-4709 or 301-827-1800
Internet: http://www.fda.gov/cber/guidelines.htm
or
Office of Communication, Education, and Radiation Programs
Division of Small Manufacturers, International, and Consumer Assistance (DSMICA), HFZ-220
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Rockville, MD 20850
Phone: 800-638-2041 or 240-276-3150
Internet: http://www.fda.gov/cdrh/guidance.html
Email: dsmica@fda.hhs.gov
Fax: 240-276-3151

Table of Contents

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document provides to you, sponsors, recommendations about certain information that should be included in an investigational device exemption (IDE) or investigational new drug application (IND) for a product intended to repair or replace knee cartilage. For the purposes of this document, a product intended to repair or replace knee cartilage, as with other articular cartilage repair or replacement products,[1] may include a biologic, device, or combination product[2] whose components would be individually regulated by the Center for Devices and Radiological Health (CDRH) and the Center for Biologics Evaluation and Research (CBER).[3,4]

This guidance supplements recommendations regarding IDE and IND submissions contained in other FDA publications (e.g., "Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated ex vivo and Intended for Structural Repair or Reconstruction" (Ref. 1)). For general information on IDEs and INDs, see http://www.fda.gov/cdrh/devadvice/ide/index.shtml and http://www.fda.gov/cber/ind/ind.htm, respectively.

[1] Prostheses such as unicondylar or total knee implants are beyond the scope of this guidance. Meniscus replacement products—which are being studied for use in preventing cartilage damage—are also beyond the scope of this guidance unless manufacturers propose new indications related to cartilage repair, replacement, or preservation.

[2] A combination product is comprised of two or more different types of regulated constituents (i.e., drug-device, drug-biologic, device-biologic, or drug-device-biologic). See Title 21 Code of Federal Regulations (CFR) 3.2(e) for further information on how combination products are defined by FDA.

[3] Forward specific questions regarding the jurisdiction over a combination product to the Office of Combination Products (OCP) at 301-427-1934 or combination@fda.gov. Information about the Request for Designation (RFD) program and guidance related to the regulation of combination products are available at the OCP website (http://www.fda.gov/oc/combination). Forward questions regarding the applicability of specific regulations for articular cartilage repair or replacement products, for which jurisdiction has already been determined, to the Center with jurisdiction.

[4] Human cells, tissues, and cellular and tissue-based products (HCT/P's) regulated solely under section 361 of the Public Health Service Act (42 U.S.C. 264) and 21 CFR Part 1271 are beyond the scope of this guidance.

We, FDA, typically regard investigational devices for articular cartilage repair or replacement to be significant risk devices (see 21 CFR 812.3(m)(1)). Therefore, if you intend to conduct clinical studies of these devices in the United States, you will likely need to submit to FDA an IDE (21 CFR 812.20(a)). All investigational studies for cellular therapy products, except for HCT/Ps that meet the criteria specified in 21 CFR 1271.10(a), including products for articular cartilage repair or replacement, require submission of an IND (21 CFR 312.20). When an IND or IDE is required, you must comply with FDA's IND regulations (21 CFR Part 312) or IDE regulations (21 CFR Part 812), as appropriate, to proceed with clinical investigations of these products. Institutional review board (IRB) approval alone is generally not sufficient to commence a clinical study in human subjects involving articular cartilage repair or replacement products (21 CFR 56.103).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

We prepared this guidance to address issues that may arise in the development of articular cartilage repair or replacement products. This guidance also reflects input received from the public and the Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) at the March 3 to 4, 2005, CTGAC meeting (Ref. 2).

In addition, we carefully considered the relevant statutory criteria for FDA decision-making and any possible burden you may incur in your attempt to address the issues and follow our recommendations in the guidance. We believe that we have considered the least burdensome approach to resolving the issues presented in this guidance document. If, however, you believe that there is a less burdensome approach, we recommend that you follow the procedures outlined in the "Guidance for Industry: A Suggested Approach to Resolving Least Burdensome Issues" (Ref. 3).

III. PRODUCT DESCRIPTION

For products subject to the IDE submission requirement in 21 CFR Part 812, you should, and in some cases are required to, provide in an IDE the following information to describe the investigational device. Depending on the particular design of the product, additional information may be appropriate:

- A complete written description of the individual components and how any components interact. See 21 CFR 812.25(d) and 812.20(b)(2).
- A description of the material(s) and any voluntary material standard(s) to which the material(s) conform. See 21 CFR 812.25(d) and 812.20(b)(2). Depending on the material, we may recommend biocompatibility testing, as described in section VI.
- A description of anticipated changes to the system. See 21 CFR 812.25(d) and 812.20(b)(2).
- A list of all instruments unique to the implantation of the product, the material and voluntary material standard to which they conform, and supporting magnified sketches or photographs of them. See 21 CFR 812.25(d) and 812.20(b)(2).

For any concurrent control product or treatment, we recommend that you provide a written description, any available drawings and photographs, and information regarding materials from which the control product is manufactured.

For products regulated under an IND, we recommend that you incorporate a description of the product into the Chemistry, Manufacturing, and Controls (CMC) section of the IND submission as described in the final guidance listed below in section IV.B. and, when finalized, the two draft guidances listed in section IV.B.

IV. MANUFACTURING AND CMC INFORMATION

A. Device Component

Under 21 CFR 812.20(b)(3), you must provide a description of the methods, facilities, and controls used for the manufacture, processing, packing, storage, and, where appropriate, installation of the device, in sufficient detail so that a person generally familiar with good manufacturing practices can make a knowledgeable judgment about the quality control used in the manufacture of the device.

As part of that information, you should provide the following:

- basic manufacturing information regarding product design issues; and
- sterilization information for the finished device, as described in the guidance entitled, "Updated 510(k) Sterility Review Guidance K90-1; Final Guidance for Industry and FDA" (Ref. 4).

B. Cellular or Gene Therapy Product or Cellular Component of Combination Product

For a cellular or gene therapy product or cellular constituents of a combination product, we recommend that you refer to "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy" (Ref. 5). When finalized, we also recommend that you refer to the following draft guidances:

- Draft "Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs)" (Ref. 6); and
- Draft "Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)" (Ref. 7).

V. NONCLINICAL DATA AND TESTING

You should provide nonclinical data sufficient to establish a scientific rationale for clinical investigation of your product, and to demonstrate an acceptable safety profile of your product prior to initiating a human clinical study (see 21 CFR 312.23(a)(8) for IND-specific requirements relating to the submission of pharmacology and toxicology information). These data can be derived from animal studies, mechanical testing, or a combination of both. You should choose the most appropriate testing to demonstrate the activities and address the safety issues raised by your product. We encourage you to design testing strategies that combine animal and mechanical testing in single studies if such a strategy does not compromise the validity of the measurements, or the usefulness of the data.

A. Animal Data and Testing

Generally, animal studies are used to assess the following issues:

- Biological response to products (e.g., biological activity [proof of concept and safety data] of each component of a combination product). You can use animal studies to demonstrate that a product's components have the potential to contribute to the clinical efficacy of the final product.
- Durability of the response (e.g., length of time needed to assess repair of the cartilage lesion and durability of the repair). You can assess durability of the response in large animal studies. Generally studies of one year in length are recommended to provide an adequate period for completion of healing and assessment of durability.
- Toxicology (e.g., potential for local and systemic toxicities due to component of the product). Local toxicities may be due to interactions of the product with the components of the joint, or degradation of the product in the joint. Systemic toxicities may be due to cell migration outside of the articular space. Potential for tumorigenicity or inappropriate differentiation of cellular products exist within or outside of the articular space.
- Dose response (e.g., effect of variation in cell number or size of lesion). Dose response can be assessed in large animal studies.

1. Suitability of animal model(s)

We recognize that choosing and determining the suitability of an animal model(s) for evaluation of any specific product is difficult because there is no perfect animal model of articular cartilage injury. As discussed at the March 2005 CTGTAC meeting (Ref. 2):

- the scientific literature contains descriptions of numerous methods for evaluating the nonclinical behavior of native cartilage and, consequently, articular cartilage repair or replacement products;
- not all of these methods may apply to a specific articular cartilage repair or replacement product; and
- goats, sheep, and horses are the most frequently used large animal models for cartilage repair.

Any of these animal species may be appropriate in studies designed to support the activity and safety of your cartilage repair or replacement product. However, we recommend that you choose the species after carefully considering the model's ability to reflect the intended clinical use.

In the case of a product containing human cells, studies performed in animals often require the use of either immunosuppressive agents to avoid rejection of the product, or the use of analogous cellular products in animals. Analogous cellular products are cellular products derived from the animal species used for testing that are analogs of the ultimate clinical product in phenotype and biologic activity. You should characterize the level of analogy with the human product in preliminary studies prior to conducting a pivotal toxicology study with the analogous cellular product.

We recommend the use of pilot studies designed to confirm the suitability of testing a particular product in a specific animal species. Several different animal studies and/or species may be necessary to adequately model functional aspects and potential toxicities of a single product. However, the number of studies needed should be determined by relevant structural and biological characteristics of the product, not by the number of components of the product. We recommend that you design nonclinical testing of cartilage repair and replacement products that contain a cellular or gene therapy component, following the principles provided in section VIII of the "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy" (Ref. 5).

Because a recommendation for a set of specific evaluations is not possible without detailed description of the articular cartilage repair or replacement product, reference is made to the American Society for Testing and Materials (ASTM) F2451-05, "Standard Guide for *in vivo* Assessment of Implantable Devices

Intended to Repair or Regenerate Articular Cartilage," approved April 1, 2005.[5] This standard provides guidelines related to the development of animal models and mechanical testing, and we recommend that you consult this standard or the applicable scientific literature when designing animal studies. Specifically, the standard contains a:

- comparison of animal models, articular cartilage defect types, and articular cartilage defect locations;
- discussion of articular cartilage defect preparation;
- description of gross and histological assessments; and
- description of various mechanical evaluations and their applicability.

2. Animal report(s) to be submitted

You should provide complete reports of any animal studies conducted using the investigational product, whether adverse or supportive, relevant to the evaluation of the safety or effectiveness of the investigational product. For INDs, you must provide a full tabulation of data suitable for detailed review for each toxicology study that is intended primarily to support the safety of the proposed clinical investigation (21 CFR 312.23(a)(8)(ii)(b)). For each nonclinical laboratory study subject to the good laboratory practice (GLP) regulations under 21 CFR Part 58, you must include a statement that the study was conducted in compliance with the GLP regulations, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance (21 CFR 312.23(a)(8)(iii) for INDs and 21 CFR 812.27(b)(3) for IDEs). You should specify in the animal report the purpose of the study and provide a detailed methods section, to include the creation and location of the cartilage defect, and supporting pathological, histological, and radiological evaluations. In addition, you should describe any differences between the product used in the animal studies and the product proposed for clinical use in the IDE or IND.

B. Mechanical Data and Testing

You should provide mechanical data for all articular cartilage repair products or a rationale addressing why mechanical testing is not necessary to establish an acceptable safety profile of the investigational product.

The mechanical testing appropriate for your product may depend on the design, material, method of attachment to the subchondral bone and/or surrounding intact cartilage, and patient indication. However, you should generally provide mechanical testing results to address the ability of the implant to withstand expected in vivo static and dynamic loading (e.g., compression, shear, propensity to generate wear debris, analysis of fixation

[5] The referenced document is an American Society for Testing and Materials Standard. The standard is available at http://www.astm.org, or contact ASTM Customer Service at service@astm.org.

method). We recommend that you compare the properties of the repaired or regenerated cartilage to those of normal articular cartilage. You should determine the aggregate modulus (H_A), Poisson's ratio (ν) and permeability (κ) of the solid phase. Permeability and aggregate modulus can be determined by confined compression creep testing, while all three of these properties can be determined from creep indentation tests using porous indentors (ASTM Standard F2451-05 contains information regarding suggested test methods). You should also include an assessment of the degree of cartilage breakdown. This may be done visually after staining with India ink or indentation probe "stiffness" evaluations.

We realize that some types of products are not capable of fully withstanding applied loads at the time of implantation (e.g., a cellular product held in place by a periosteal flap or a flexible scaffold that will eventually be populated by cells that ultimately form a load-bearing tissue). For these products, it would be appropriate to characterize various properties at discrete timepoints. You should initially assess the product's ability to maintain its location within the loaded joint, and subsequently continue to assess this characteristic while adding assessments of the newly-formed tissue and its ability to bear applied loads.

When there are differences between the proposed clinical product and the product tested, you should explain how or why the results are relevant in establishing the relative safety of the proposed product. Regardless of the evaluations which are performed, you should compare the properties of the repaired or regenerated tissue to control tissue (e.g., the cartilage collected from an unoperated control joint). While it is understood that the repair tissue might have properties that differ from those of normal cartilage, you should describe why these differences might not be relevant to the in vivo and clinical behavior of the product.

You should provide complete reports of any mechanical testing conducted on the investigational product, whether adverse or supportive, that are relevant to the evaluation of the safety or effectiveness of the investigational product. Each test report should include, but need not be limited to, the following elements:

- identification of the components that comprised the product tested;
- the set-up;
- the procedures;
- rationale supporting the testing environment as being a worst case condition
- rationale for the loading modes chosen;
- the results; and
- a discussion of the results in terms of the expected in vivo and clinical performance of the system.

You should also provide a comprehensive summary of all mechanical testing in addition to complete reports for each test.

VI. BIOCOMPATIBILITY

Depending on the material(s) used in the product, we may recommend biocompatibility testing. FDA's guidance entitled, "Use of International Standard ISO-10993, 'Biological Evaluation of Medical Devices Part-1: Evaluation and Testing'" (Ref. 8) and/or ASTM F748-04, "Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices"[6] may be recommended as acceptable approaches for conducting biocompatibility testing. You should include in the IND or IDE a complete test report describing the tests performed, the specific methods utilized, and the results.

In addition, for any biological or drug component (e.g., bone morphogenic protein, bovine protein), we recommend that you follow any applicable FDA guidances.

VII. CLINICAL STUDY PROTOCOLS

Clinical studies of articular cartilage repair or replacement products must be conducted in compliance with IDE regulations (21 CFR Part 812) or IND regulations (21 CFR Part 312), along with Informed Consent (21 CFR Part 50) and IRB regulations (21 CFR Part 56) and other applicable regulatory requirements.

A. Design

In general, the clinical development program for an investigational knee cartilage repair or replacement product should proceed through an orderly series of exploratory and confirmatory clinical studies. The number of clinical studies as well as the specific design requirements for each of these studies is contingent upon multiple factors, including the characteristics of the investigational product, the route of product administration, the characteristics of the target patient population and the proposed product indication. Consequently, this guidance provides only a broad outline of the major features to consider in designing a clinical study.

1. Exploratory Clinical Studies

You should design exploratory clinical studies that are conducted early in clinical development to obtain, in addition to any other features, the following information:

- safety data;
- data assessing the ability to properly administer the product, including identification of any study procedures that should be modified to optimize product administration;

[6] Id.

- bioactivity data, such as assessments of cartilage integrity based upon imaging results and biopsy findings;
- data assessing the appropriateness of the target patient population; and data providing information concerning the activity of the product in vivo or other information related to product activity that may be informative for future development such as:

    - product dose-response relationships
    - product design-response characteristics

You should comprehensively evaluate exploratory clinical study data to facilitate the design of confirmatory studies. At the conclusion of exploratory clinical studies, you should be able to provide clinical data explaining the important aspects of the proposed confirmatory clinical studies that apply to the investigational product, such as:

- data that support the product dose and design characteristics;
- route of administration, including surgical technique in the use of the product;
- extent and nature of follow-up evaluations;
- study subject sample size;
- eligibility and ineligibility criteria;
- choice of the major study endpoints; and
- statistical assessments of the major study endpoints.

An important consideration for an exploratory clinical study of knee cartilage repair or replacement products is the use of a control group(s) to optimize the interpretability of the exploratory findings. In general, the most important clinical outcomes associated with use of these products are relief of pain and restoration of knee function, outcomes we believe are highly susceptible to bias due to assessment subjectivity. The use of control groups may greatly facilitate the interpretation of the clinical study findings, even if–because of the nature of the studies–the statistical assessments lack the robustness or power expected of confirmatory clinical studies.

2. Confirmatory Clinical Studies

Confirmatory clinical studies are designed to obtain hypothesis-testing data (i.e., to test a primary efficacy hypothesis and provide sufficient supportive data for that hypothesis as well as corresponding safety data). Depending upon the characteristics of the investigational product, safety concerns may render a larger sample size appropriate than one might estimate based solely upon the size of the projected primary efficacy endpoint treatment effect. Consequently, we recommend that you consider both efficacy and safety considerations in designing confirmatory clinical studies.

Typically, confirmatory clinical studies utilize a randomized, controlled design. Whenever possible, we recommend that you utilize a randomized, controlled study design with endpoints ascertained in a blinded manner (e.g., primary endpoints should be performed in either a completely blinded manner or with the use of major endpoint evaluators who are blinded to the study treatment assignments). However, alternative confirmatory study designs may be considered; as described, for example, in existing FDA guidance for products regulated under IND.[7] You should provide us with data (from your studies and applicable literature) and a rationale to support your confirmatory study design prior to initiation of a confirmatory study for any cartilage repair product.

Listed below in section VII.B through G are important considerations for the design of both exploratory and confirmatory clinical studies.

B. Control Group

Multiple options exist for the choice of a study's control groups, and we recommend that you review the "Guidance for Industry: E10 Choice of Control Group and Related Issues in Clinical Trials" (Ref. 9). This guidance, while intended for biological products and drugs, contains concepts which, we believe, may also be relevant to the clinical study of an investigational device-biologic combination product.

In general, control groups may be broadly divided into either concurrent or historical controls. Rapid advances in surgical techniques and the medical care of damaged knees over the past several years suggest that you should generally use a concurrent control group to obtain the most informative clinical data. We believe historical controls are rarely sufficient for confirmatory clinical studies of knee cartilage repair or replacement products.

The most common types of concurrent control groups include placebo controls, sham-surgery controls, active-comparator controls, or standard care controls. If you choose an active comparator control, we recommend that you use one that is well accepted as standard treatment for the indication. For example, this comparator may be an approved or licensed product or a well-accepted surgical procedure for the indicated condition. Comparator procedures may include the following: microfracture, debridement, osteochondral autograft transplantation (e.g., mosaicplasty), autologous chondrocyte implantation, autogenous perichondral or periosteal grafts, and osteochondral allografts, depending on the standard treatment for the indication.

[7] For cell, gene therapy, and combination products regulated under section 351 of the Public Health Service Act (42 USC 264), please refer to the discussion of surrogate endpoints in FDA's "Guidance for Industry on Fast Track Drug Development Programs: Designation, Development, and Application Review" dated January 2006 (http://www.fda.gov/cber/gdlns/fsttrk.pdf).

You should provide a rationale for the selected comparator(s). This rationale should include the comparability of the experimental and control treatments with respect to the extent of the surgical procedures involved as well as the duration and extent of rehabilitation.

A study could also include more than one comparator study arm. For example, a controlled study could compare treatment effects across a range of investigational product dosages or compare treatment effects among a group of alternative procedures/products.

"Sham controlled studies" represent one study design and choice of control group which may allow for discrimination of patient outcomes caused by the test treatment from outcomes caused by other factors such as patient or observer expectations. This type of study design could be considered in studies with subjective endpoints such as reduction in patient-reported symptoms. Sham surgical procedures/treatments involve more risk than the placebo control arm in drug trials and should be used in limited circumstances. This study design should only be considered when it is methodologically necessary, i.e. when designs that are unblinded are methodologically unacceptable (e.g., because endpoints are subjective) and when a "no treatment" control is methodologically required. Furthermore, the withholding of treatment should not lead to serious harm, such as death or irreversible morbidity. FDA recognizes that it may be difficult for sponsors to develop a clinical study design with a sham control arm that investigators, institutional review boards, and patients believe is ethical; for this reason, studies involving a sham control arm should be carefully considered and planned. Additionally, if a sham procedure/treatment is being considered in a clinical investigation involving children, the requirements of 21 CFR Part 50 Subpart D (Subpart D) also apply.

We recommend that, for most studies, randomized controls be used such that the control group populations have lesions that are similar to the experimental group in terms of depth, size, and extent of cartilage/bone damage.

C. Patient Population

We recommend you prespecify the following patient selection characteristics within a study protocol's eligibility criteria:

- degree of pain;
- presence or absence of osteoarthritis and method of diagnosis of osteoarthritis;
- minimum and/or maximum degree of physical function;
- location of articular lesion (e.g., medial femoral condyle, lateral femoral condyle);
- depth of lesion;
- size area of lesion (i.e., in cm^2);
- concomitant joint pathology (e.g., meniscal tear, ligament tear); and
- whether there has been prior treatment for the lesion.

In defining each of these characteristics, you should select unambiguous definitions, preferably based upon well-accepted evaluation techniques. One acceptable way for determining subject eligibility by size and extent of the cartilage lesion is through use of the International Cartilage Rating System (ICRS), as described in the International Knee Documentation Committee (IKDC) Knee Examination Form-2000.[8] You should provide a scientific rationale in your study protocol or supportive documents for selecting minimum values, maximal values, lesion depth, and lesion size. To determine subject eligibility by clinical parameters such as pain and clinical function we recommend that you use an established clinical measurement instrument such as those described in section VII.D.

D. Study Endpoints

We recommend that clinical studies assess the endpoints described in this section. However, the applicability of these endpoints depends on the characteristics of the investigational product and its method of administration.

We believe that clinically meaningful endpoints, such as improvement in pain and physical function, provide the most persuasive evidence of efficacy. Consequently, you should identify changes in pain and/or physical functioning as the primary endpoint for confirmatory clinical studies. Examples of measures that may be used to assess these endpoints include the:

- Knee injury and Osteoarthritis Outcome Score (KOOS);
- IKDC Subjective Knee Evaluation Form-2000;
- Cincinnati Knee Rating System;
- Symptom Rating Form; and
- Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC).

Depending on the primary abnormality in the target population and other study design characteristics, we recommend that you use change in knee pain and/or physical functioning as the single primary endpoint in confirmatory studies. If you use a co-primary approach, then statistical success should be met in both endpoints in a manner that preserves the overall type 1 error.

Secondary endpoints that may be studied include:

- arthroscopic assessments of changes in the size, location, and grade of cartilage lesions both before and after debridement, if debridement is intended. One acceptable method for assessing these endpoints is through use of the ICRS, as described previously in section VII.C above.

[8] This form is contained in the ICRS Cartilage Injury Evaluation Package, available at http://www.cartilage.org/_files/contentmanagement/ICRS_evaluation.pdf.

- assessment of the physical findings from examination of the knee joint, including:

    - both passive and active range of motion
    - quadriceps muscle strength
    - presence of patellar subluxation
    - presence and degree of effusion
    - alignment
    - presence and degree of crepitus
    - presence and degree of ligament laxity

- arthroscopic evaluation to assess:

    - the integrity of repaired tissue
    - the binding of implanted investigational product to adjacent tissue, including assessments of stiffness/firmness based upon tissue probing

- histologic evaluation at both short (e.g., six months) and long term (e.g., two years) follow-up in a subset of subjects to assess:

    - matrix zonal organization
    - cell density
    - cell morphology (i.e., chondrocytic vs. fibroblastic)
    - type I or type II collagen concentration
    - Aggrecan concentration, size, and composition
    - Dermatan sulfate proteoglycan concentration
    - noncollagenous protein concentrations (fibronectin, tenascin)
    - inflammatory response

- serological assessments for antibody formation and evidence of inflammation.
- assessment of synovial fluid samples for cell count, sterility and, as applicable, markers of inflammation and antibody formation.
- joint/cartilage structure as assessed by magnetic resonance imaging (MRI), for:

    - articular surface integrity
    - thickness and volume of chondral surface
    - subchondral bone plate contour
    - thickness and volume of synovial membrane
    - volume of synovial fluid

We recommend that the protocol specify which MRI techniques and views will be taken, and that the images be interpreted by at least two independent (blinded) readers. The protocol or study supportive documents should include a clear, prospectively stated, description of the plan for review of these images, and plans for resolving conflicting readings.

E. Investigational Product Administration

The clinical protocol and supportive documents must provide a detailed description of the procedures to be used in administration of the investigational product. See 21 CFR 312.23(a)(6) and 812.25(b). This description is especially critical in multi-center studies. We acknowledge that many surgical procedures use techniques common to standard surgical practice and these procedures can be briefly summarized in the description of the investigational product administration procedures. Any unique procedures for administration of the investigational product should be described in detail.

For plans related to any surgical procedures, the clinical protocol should identify and provide details on the:

- **Surgical technique** for both the investigational and control treatments, including the type of anesthesia, the size of the incision, and the use of antibiotics and pain medications, as applicable. We recommend that the surgical procedures be comparable, as much as possible, between treatment groups.
- **Plans for post-operative care**. Supportive documents should address the use of continuous passive motion; the duration, method, and frequency of weight bearing; the type, dose, and frequency of pain medication used; and the type and frequency of rehabilitation. These factors should be standardized between/among treatment groups when possible.

F. Follow-Up

You should include sufficient follow-up information for all investigational products within a premarket approval application (PMA) or BLA. For investigational products which are resorbed, degraded, or remodeled, the study subject follow-up duration should be based on information gathered from in vivo and in vitro nonclinical studies, as well as from information based upon the natural history of the underlying, target clinical condition. However, even in this situation, we recommend that the PMA or BLA include two-year follow-up safety information on a subset of study subjects (this subset of subjects could be from initial, exploratory clinical studies). Data from an extended follow-up period provides an important component of the information to be contained within product labeling. Therefore, the subjects enrolled in initial or exploratory studies should continue to be followed during the period of confirmatory studies so that you ultimately provide some long-term follow-up information from these initial studies. For reference, guidance on the length of follow-up for gene therapy products is available in

the "Guidance for Industry: Gene Therapy Clinical Trials - Observing Participants for Delayed Adverse Events" (Ref. 10).

For investigational products which are not reabsorbed or degraded, a longer duration of patient follow-up is recommended to document safety outcomes. In this situation, generally five years of patient follow-up is recommended. This may be initiated during the pre-market phase and continued post-market.

G. Adverse Event (Risk) Reporting

This section concerns adverse event (AE) reporting by the investigator(s) to you. See 21 CFR 312.64 and 812.150(a)(1).[9] When an investigator reports AEs to you, the investigator should stratify the AEs by those general to any surgery, those related to knee surgeries (open vs. arthroscopic), and those specific to the investigational product. We recommend that you incorporate definitions or descriptions of known or anticipated AEs into the case report forms (CRFs) to ensure uniform reporting. You should also state in the protocol and CRFs that all subsequent surgical interventions, investigational product-related or not, should be reported and recorded.

We define subsequent surgical interventions as follows:

- Revision - a procedure that adjusts or in any way modifies or removes part of the original investigational product, with or without replacement of a component; it may include adjusting the position of the original investigational product. If the investigational product is used/implanted in conjunction with an FDA approved product/component, a revision to any component, even to the approved component, should be reported as a revision.
- Removal - a procedure where all or part of the original investigational product is removed with or without replacement.
- Reoperation - any subsequent surgical procedure at the involved surgery site that does not involve removal, modification, or addition of any component(s) to the product.

[9] For requirements regarding adverse event reporting by the sponsors to FDA, see 21 CFR 312.32 and 812.150(b)(1).

VIII. REFERENCES

1. Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated ex vivo and Intended for Structural Repair or Reconstruction, May 1996 (http://www.fda.gov/cber/gdlns/gdexv.pdf).

2. Cellular, Tissue, and Gene Therapies Advisory Committee meeting, March 3, 2005 (http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4093T1.htm); March 4, 2005 (http://www.fda.gov/ohrms/dockets/ac/05/transcripts/2005-4093T2_01.htm).

3. Guidance for Industry: A Suggested Approach to Resolving Least Burdensome Issues, September 2000 (http://www.fda.gov/cdrh/ode/guidance/1188.html).

4. Updated 510(k) Sterility Review Guidance K90-1; Final Guidance for Industry and FDA, August 2002 (http://www.fda.gov/cdrh/ode/guidance/361.html).

5. Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy, March 1998 (http://www.fda.gov/cber/gdlns/somgene.pdf).

6. Draft Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs), August 2003 (http://www.fda.gov/cber/gdlns/cmcsomcell.pdf).*

7. Draft Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs), November 2004 (http://www.fda.gov/cber/gdlns/gtindcmc.pdf).*

8. Guidance on Use of International Standard ISO-10993, "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing," May 1995 (http://www.fda.gov/cdrh/g951.html).

9. Guidance for Industry: E10 Choice of Control Group and Related Issues in Clinical Trials, May 2001 (http://www.fda.gov/cber/gdlns/clincontr0501.htm).**

10. Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events, November 2006 (http://www.fda.gov/cber/gdlns/gtclin.pdf).

*These draft guidances, when finalized, will represent FDA's current thinking on their respective topics.
**International Conference on Harmonization document.

European Medicines Agency

London, 17th September 2009
Doc. Ref. EMEA/CAT/CPWP/288934/2009

COMMITTEE FOR ADVANCED THERAPIES (CAT)

DRAFT for External Consultation

REFLECTION PAPER ON

IN-VITRO CULTURED CHONDROCYTE CONTAINING PRODUCTS FOR CARTILAGE REPAIR OF THE KNEE

DRAFT AGREED BY CPWP	July – September 2009
DRAFT AGREED BY CAT	July - September 2009
DRAFT DISCUSSED BY BWP, EWP, SWP, SAWP	September 2009
ADOPTION BY CAT FOR RELEASE FOR CONSULTATION	11 September 2009
END OF CONSULTATION (DEADLINE FOR COMMENTS)	31 December 2009[1]
AGREED BY <WORKING PARTY>[2]	<month year>
ADOPTION BY CAT	<day month year> [3]

Comments should be provided using this template to AdvancedTherapies@emea.europa.eu

KEYWORDS	*Chondrocytes, Cell therapy, autologous, Advanced Therapies, Cartilage repair, Quality, Nonclinical, Clinical*

[1] Last day of the month concerned
[2] If other WPs have been involved in discussions this needs to be specified
[3] Last day of relevant Committee meeting

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 75 23 70 51
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

13

14 **REFLECTION PAPER ON**

15 ***IN-VITRO* CULTURED CHONDROCYTE CONTAINING PRODUCTS FOR CARTILAGE**
16 **REPAIR OF THE KNEE**

17 **TABLE OF CONTENTS**

22

23

1. INTRODUCTION (background)

This reflection paper addresses specific points related to products containing autologous chondrocytes intended for the repair of lesion of cartilage of the knee not discussed in the 'Guideline on human cell-based medicinal products' (EMEA/CHMP/410869/2006) and therefore it should be read in conjunction with the guideline.

2. DISCUSSION

CONSIDERATIONS ON QUALITY DATA

For novel products as well as for products with clinical experience gathered before entry into force of Reg. No. (EC) 1394/2007 the same level of quality is expected for a central marketing authorisation application.

Starting material

The active substance is based on chondrocytes obtained from a cartilage biopsy. Due to dedifferentiation tendency of the chondrocytes when cultured in monolayer, the yield in cell number is limited by the size of the biopsy and will limit the size of the defect that can be treated with the resulting product. Therefore specific consideration should be given to the amount and quality of the starting material to ensure that sufficient cell numbers can be produced for the presented defect to be treated.

The collection of the cartilage biopsy should be standardised in order to minimise possible contaminants (fibroblasts) arising from fragments of the synovial membrane. The presence / absence of fibroblasts should be controlled through appropriate in-process testing. Acceptance criteria in relation to cellular impurities should be set through process validation.

Manufacturing process

The total number of cells to return to differentiated state depends on the number of duplication in monolayer culture, thereby limiting the overall expansion of the biopsy. Therefore adequate limits to population doubling / passage number should be set considering appropriate functional markers related to the differentiation stage and the resulting cartilage forming capacity of the cells.

In cases where a 3-dimensional cell culture process in combination with a structural component is used, attention should be paid to the functionality and number of cells in the combination product, and not only of the cell suspension.

Process validation is a prerequisite to ensure consistent manufacture. Given the limitations related to the cellular material available (especially for autologous products) for process validation, alternative material with comparable characteristics could be used e.g. collected from joint replacement surgery.

Potency

Two main aspects for the biological characterisation and control of chondrocytes containing products are the cartilage forming capacity and stage of differentiation of the cells. Potency can be expressed through (a) functional assay(s) established for characterisation of the product and for process validation. The functional assay is expected to be suitable to detect changes in the product in relation to the aspects described above which may be clinically meaningful.

Due to time constraints, for batch release, an assay based on surrogate marker(s) could be envisaged. In case mRNA based assays or other surrogate markers are used, their correlation with a functional assay is expected.

Quality controls

Biocompatibility of all materials coming into contact with the cells should be demonstrated. This includes not only materials used during the manufacturing process, but also those used as part of the application (e.g. membranes for local containment, fibrin glues).

CONSIDERATIONS ON NON-CLINICAL DATA

Clinical experience gathered prior to entry into force of Reg. No. (EC) 1394/2007 can be considered on a case-by-case basis. Clinical experience might substitute for some parts of the non-clinical development. However, the acceptability of such approach will clearly depend on the quality of the data that have been collected. Such approaches have to be justified by the Applicant and are at the Applicant's risk. Of high importance are, as part of such justification, what changes have been made to the manufacturing process over time, and what impact these had, i.e. it needs to be justified that the data submitted to substitute for non-clinical data are indeed relevant to the product which is applied for. In any case, justification for the omission of any non-clinical analyses has to be provided[0].

Pharmacology

Initial proof of principle studies could be initiated with the use of *in vitro* cell culture methods such as 3-dimensional cell culture models (i.e. Pellet culture model, 3-dimensional alginate cell culture). Attention should be paid to use of the final product in the proof of principle animal studies. This includes the use of the proposed cell-device combination and other non-cellular components (e.g. membranes, fibrin glues), where appropriate.

First *in vivo* proof of principle studies can be conducted in small animal models where, usually, data can be generated relatively quickly with a larger sample size. An example could be the ECFA model, in which human chondrocytes are implanted ectopically in immuno-compromised animals. However, such models have limitations, e.g. the different anatomical structure of the knee joint, or difficulties of manipulation and mimicking the clinical use.

As immuno-compromised large animal models are not available it is recommended to use autologous animal cells. The pivotal non-clinical study should be conducted in a large animal model to mimic as much as possible the situation in humans and to allow for more invasive testing than possible in humans. Currently the best available large animal models are goat, horse or sheep. Mouse models will normally not be sufficient as a proof of concept. Deviation from these principles should be justified.

The pivotal non-clinical studies should be long enough to show regeneration and repair and to obtain enough evidence for a long term clinical use in humans. These studies could include testing for biomechanical properties and tissue integrity (morphological characteristics of the cartilage). The number of animals in these studies should allow robust analysis of the data.

The quality of animal cells should be comparable to the medicinal product for clinical use. The impact of deviations in the manufacturing process used for the animal cells on quality should be justified.

Biodistribution

Biodistribution studies in a relevant animal model are considered necessary in cases where the product might not be sufficiently physically retained, e.g. by a membrane and/or when a scaffold is not applied together with a physical barrier. In any case, potential biodistribution can be of clinical concern, and thus the Applicant should justify their approach to show absence or lack of clinical significance of any untoward safety issue related to biodistribution.

Toxicology

The necessity of conventional toxicity studies depends on the nature of the product and should follow a risk-based approach.

Conventional toxicity studies may not be required for autologous chondrocyte products; safety endpoints may be incorporated into proof of concept studies in justified cases.

CONSIDERATIONS ON CLINICAL DATA

Potential claims.

The principal aim for autologous chondrocytes containing product is to repair cartilaginous defects either from traumatic damage or degenerative disease. The indication could be further defined by relevant components, particularly, number of defects treated (multiple or single defect), size of defect, localisation of the defect (such as femoral condyle or trochlea), symptomatic or asymptomatic defect, grading of the defect (such as ICRS score), and previous failed therapies (such as after failed previous therapeutic or surgical intervention). Due to different aetiologies of the lesions, separate safety and efficacy studies would be appropriate. For claims of the product as second line treatment, special attention should be paid to the characteristics of the previously treated lesion.

Subject characteristics and selection of subjects.

The patient population included in the studies should be selected by relevant criteria like symptoms, functionality, localisation, size and depth of the knee defect(s), concomitant joint pathology(ies), and previous treatments of the defect. Restriction of target population may increase precision of study (such as excluding patients with previous mosaicplasty, advanced osteoarthritis etc.) but also could diminish generalisation or benefit of the results (such as limiting the defect size).

Strategy and design of clinical trials.

A. Clinical Pharmacology.

Pharmacokinetics. As there is no clear common agreement for conventional clinical kinetic data needed to be analysed in clinical setting, the majority of the issues regarding clinical pharmacology are expected to be addressed during the non-clinical phase. If non-cellular component are present, their combination with cells is expected to be assessed clinically for compatibility, degradation rate and functionality.

Pharmacodynamics. Macroscopic, histological and MRI assessment of the repair tissue are considered adequate tools for pharmacodynamic assessment of autologous chondrocytes containing products. MRI is to date, considered clinically relevant and could be included in trial protocols, although it is acknowledged that it is not validated as such in the follow up of the repair tissue. Validation of MRI in a large animal (such as horse or sheep) with histopathological investigations might yield supportive data to surmount the clinical database (see non-clinical section).

B. Exploratory trials.

The dose definition should be carefully chosen reflecting both actual numbers of the cells engrafted and adjustments for particular defect sizes (e.g. expressed in minimal number of cells/cm^2). Parallel group, randomised, controlled studies are recommended, where comparative agent could be similar to the one used for confirmatory study and concomitant therapy could be a perisurgical, therapeutic, rehabilitation together with a follow up regimen acceptable from clinical perspective. The study duration is expected to be not less than 2 years for clinical endpoints and not less than 1 year for structural endpoints.

The published data from other relevant studies could be supportive for dose definition, provided that the quality of the product is comparable.

Dose definition could be justified also by unequivocally observed effect size (e.g. more the 10 point change in a KOOS subscale) and sufficient safety database.

Depending on the amount and quality of clinical data gathered before entry into force of Reg No. (EC) 1394/2007 exploratory studies might not be required. Justification for the omission of exploratory studies should be provided, including evidence that in case of changes in the manufacturing process over time these do not affect the clinical development program.

The clinical data should be sufficient to justify the administration procedure and the design of the confirmatory studies.

Exploratory clinical trial endpoints should be suitable to address pharmacodynamics, dose and safety.

C. Confirmatory trials.

Methods to assess efficacy.

Definition of the primary endpoints. Patient-based outcome data is acceptable as primary endpoint in the pivotal study, given the current lack of other outcome measures that are both sensitive and objective. For patient-based outcomes, validated methods to assess improvement of function and pain should be used (e.g. knee injury and Osteoarthritis Outcome Score (KOOS) or other validated outcome measures). Other primary endpoints, including either treatment failure or total joint replacement can be used, however these should be validated methods.

Definition of secondary endpoints. The structural improvement is the main secondary endpoints. The suitable structural endpoints could be chosen from blinded standardised MRI with/or without histological evaluations. Until validated methods are available, it is the Applicant's responsibility to demonstrate that the method is qualified for its intended use. Structural endpoint could also serve as a relevant supportive surrogate marker for benefit risk assessment in case of need for long-term efficacy that could be performed post-marketing.

Other specific secondary endpoints could be used e.g. the ones representing clinical / functional assessments (such as IKDC subjective scale, Lysholm score, ICRS objective scale, physical findings for the knee) or the ones representing structural assessments (such as arthroscopic and X-ray assessments).

Trial design

For patients with lesions of less than 4 cm^2 clinical non-inferiority/superiority with supporting structural superiority against currently employed reasonable surgical comparative therapy (such as microfracture) is the reasonable option.

For patients with lesions of more than 4 cm^2, no standard therapy has shown unequivocal efficacy, therefore superiority against best standard of care is the reasonable option. Medicinal product without centralised authorisation would not be a valid comparator.

For the confirmatory trials and due to the nature of the product, blinding of the trial design may be difficult to be maintained. For these trials prospective randomised, open label, blinded evaluation is recommended.

Various options can be considered for the design of confirmatory trials, e.g.

- A randomized controlled trial including microfracture as comparator. In this case the appropriateness of the microfracture procedure with respect to the lesion size to be treated needs to be addressed, since microfracture is only recommended in smaller lesions.

- A randomized controlled trial including an active comparator. If a licensed chondrocyte-containing product that has been validated in a randomized controlled trial is used as comparator, a non-inferiority design may be considered.

- A randomized controlled trial including a standardized exercise program as control arm. The standardized exercise program should be suitable to stabilize muscle function and could be viewed as an active placebo control. The design should consider a switch of patients from active placebo to the verum arm according to predefined criteria.

- Any other clinical trial design, when appropriately justified.

For larger lesions, where there is no established treatment available, a dose response assessment is desirable. This could be done by including the assessment of the dose-response relationship in the confirmatory study, whereby the dose (of chondrocytes) per size (cm2) of the defect would be added as a covariate.

Study duration. A 3 year follow-up for clinical efficacy evaluation is normally necessary. However, for registration purposes, structural repair by histological / MRI analysis could be acceptable at earlier evaluation timepoints, where appropriately justified. The follow-up period for clinical efficacy could be envisaged post-authorisation (Efficacy follow-up within Art. 14 of Reg. (EC) 1394/2007) provided positive benefit risk profile is obtained.

D. Methodological considerations

Numerous procedures and treatment related risk factors are emerging and include: (1) *Patient factors*, especially size of the defect. Other reasonable patient factors to be considered are BMI, gender, age, sports activity, and defect localisation; (2) *Variability due to other therapies*, such as variability of surgical procedures among different centres and surgeons (standardised surgical protocols should be done); symptomatic treatment allowed (both as pre-procedurally or peri-procedurally prior the implantation), peri-surgical procedures (such as arthroscopy or open surgery procedures prior the implantation), rehabilitation protocols and the follow-up programs are reasonable to be considered. These considerations demonstrate that a standardized approach might be valuable in order to reduce variability between study arms that could render interpretation of data difficult.

At best the most important factors should be identified beforehand and be taken into consideration by proper stratification of the randomisation and/or inclusion of these factors into the analysis model by prospectively planned subgroup analyses.

Clinical safety evaluation

General safety issues. The autologous chondrocytes-containing products have been used for more then 15 years in clinical practice and the experience for this class of products is relevant and has to be considered. For the safety assessment, the clinical program could consider results of quality and non-clinical investigations as well as unresolved issues that could not have been assessed non-clinically.

For products for which clinical data has been gathered before entry into force of Reg No. (EC) 1394/2007, the acceptability of safety data will depend on the quality of the data and their collection over the years.

Specific safety issues. Special attention has to be paid on long-term structural changes, such as local histological or MRI detectable changes, rates of treatment failures, as defined through relevant investigation techniques, including re-operation for revision purposes. In cases of treatment failure, a root-cause analysis should be performed in order to identify the factors, which gave rise to treatment failure (i.e. quality of the product, surgical procedure, patient characteristics).

3. CONCLUSION

4. REFERENCES

Guideline on human cell-based medicinal products **(**EMEA/CHMP/410869/2006**).**

Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (OJ L 324 of 10.12.2007, p 121)

ANNEX I

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

ChondroCelect 10,000 cells/microlitre implantation suspension

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

2.1 General description

Characterised viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins.

2.2 Qualitative and quantitative composition

Each vial of product contains 4 million autologous human cartilage cells in 0.4 ml cell suspension, corresponding to a concentration of 10,000 cells/microlitre.

For a full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

Implantation suspension
Before re-suspension the cells are settled to the bottom of the container forming an off-white layer and the excipient is a clear colourless liquid.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Repair of single symptomatic cartilage defects of the femoral condyle of the knee (International Cartilage Repair Society [ICRS] grade III or IV) in adults. Concomitant asymptomatic cartilage lesions (ICRS grade I or II) might be present. Demonstration of efficacy is based on a randomised controlled trial evaluating the efficacy of Chondrocelect in patients with lesions between 1-5cm².

4.2 Posology and method of administration

ChondroCelect must be administered by an appropriately qualified surgeon and is restricted to hospital use only. ChondroCelect is solely intended for autologous use and should be administered in conjunction with debridement (preparation of the defect bed), a physical seal of the lesion (placement of a biological membrane, preferentially a collagen membrane) and rehabilitation.

Posology
The amount of cells to be administered is dependent on the size (surface in cm²) of the cartilage defect. Each product contains an individual treatment dose with sufficient number of cells to treat the pre-defined lesion size, as measured at biopsy procurement. The recommended dose of ChondroCelect is 0.8 to 1 million cells/cm², corresponding with 80 to 100 microlitre of product/cm² of defect.

Elderly
Limited data are available on adult patients older than 50 years.

Paediatric population
The safety and efficacy in children and adolescents (aged less than 18) have not been established. ChondroCelect is therefore not recommended for use in children and adolescents below 18 years.

Method of administration
ChondroCelect is intended solely for use in autologous cartilage repair and is administered to patients in an Autologous Chondrocyte Implantation procedure (ACI).

Implantation of ChondroCelect is to be performed during arthrotomy under sterile conditions and requires both preparation of the defect bed and a seal (biological membrane) to secure the implant. Complete joint haemostasis must be achieved prior to membrane fixation and cell implantation. In clinical studies with ChondroCelect a periosteal flap was used as a biological membrane. Scientific publications have shown that commercially available collagen membranes can be used as an alternative to the periost in ACI procedures. However, ChondroCelect has not been evaluated in combination with collagen membranes in clinical studies, although a commercially available collagen membrane has been used in patients treated with ChondroCelect under compassionate use. The safety data obtained in these patients do not indicate a particular safety concern, and confirm a lower incidence of hypertrophy as suggested by scientific literature on the use of collagen membranes versus periost.

The implantation should be followed by an appropriate rehabilitation schedule for approximately one year, as recommended by the physician (see section 4.4).

Full technical details on the procedures associated with this implantation technique are provided in the ChondroCelect user manual.
For information on preparation and handling of ChondroCelect, please refer to section 6.6.

4.3 Contraindications

Hypersensitivity to any of the excipients or to bovine serum.
ChondroCelect must not be used in case of advanced osteoarthritis of the knee.

4.4 Special warnings and precautions for use

General
ChondroCelect is an autologous product and should under no circumstances be administered to other patients.
Patients with acute or recent history of bone or joint infections should be temporary deferred until documented recovery.

Precautions for use
Concomitant knee problems like early osteoarthritis, osteochondritis dissecans (OCD), instability of the knee, cartilage lesions at other locations than the femoral condyle, lesions of knee ligaments or of the meniscus, varus or valgus malalignment (abnormal weight distribution in the knee), and inflammatory joint disease are potential complicating factors. In the pivotal study of ChondroCelect, patients with these comorbidities of the knee were excluded from treatment. Where possible, concomitant knee problems should be corrected prior to or at the latest at the time of ChondroCelect implantation.
In the pivotal study there was no influence of Body Mass Index (BMI) on outcome but bibliographic data shows that a BMI over 30 may also adversely affect the success of the procedure.

Rehabilitation
Upon implantation, the patient should follow an appropriate rehabilitation schedule and physical activity should be resumed as recommended by the physician. Depending on the location, the size of the lesion and the patient's profile, appropriate rehabilitation instructions have been developed. Too early and vigorous activity may compromise the grafting and the durability of clinical benefit from ChondroCelect. Therefore the treated knee should be protected according to the recommendations as outlined in the appropriate rehabilitation schedule, to avoid early damage which might lead to graft failure.
Details and information on the appropriate rehabilitation schedule is provided in the ChondroCelect user manual.

Cases in which ChondroCelect cannot be supplied
In some cases it can be possible that the source chondrocytes of the patient are not expandable or that the release criteria are not met, due to poor biopsy quality, patient characteristics, or manufacturing failure. Therefore it can occur that ChondroCelect cannot be delivered. The surgeon will be informed as early in the process as possible, and should hence select an alternative treatment for the patient concerned.

4.5 Interaction with other medicinal products and other forms of interaction

Fibrin glues are routinely used in ACI procedures to seal the outside margins and to improve the water-tightness of the compartment of the biological membrane used to cover the defect. Fibrin sealant products differ significantly in their quantitative and qualitative composition. *In vitro* interaction studies were performed with a commercially available fibrin glue containing aprotinin (a fibrinolysis inhibitor of bovine origin). . These studies have demonstrated that this type of fibrin sealant can be safely used with ChondroCelect. No interaction studies with any other type of fibrin glues were performed. However, the concomitant use of another type of fibrin glue with a synthetic fibrinolysis inhibitor (tranexamic acid) in the pivotal clinical trial did not reveal any safety signal.

Pain medicinal products should be used according to the recommendations of the responsible surgeon.

4.6 Pregnancy and lactation

For autologous cartilage cells no clinical data on exposed pregnancies are available.
Conventional reproductive and developmental toxicity studies are not considered relevant, given the nature and the intended clinical use of the autologous cell therapy product.
As ChondroCelect is used to repair a cartilage defect of the knee and is implanted with the ACI procedure using open-knee surgery, it is not recommended for pregnant or breast-feeding women.

4.7 Effects on ability to drive and use machines

Due to the surgical nature of the underlying procedure, implantation with ChondroCelect has a major influence on the ability to drive and use machines. During the rehabilitation period that follows treatment with ChondroCelect, patients should refer to their treating physician and follow their advice strictly.
Driving cars and using machines may be limited during the rehabilitation period.

4.8 Undesirable effects

In a randomized, controlled study in the target population, 51 patients were treated with ChondroCelect. In these patients, a periosteal flap was used to secure the implant.
Adverse reactions occurred in 78.4% of the patients over a 36-months postoperative follow-up period. The most common adverse reactions were arthralgia (47.1%), cartilage hypertrophy (27.4%), joint crepitation (17.6%) and joint swelling (13.7%). Adverse reactions collected from 370 patients included in a Compassionate Use Program are similar to those reported in the target population.

Most of the reported adverse reactions were expected as related to the open-knee surgical procedure. The most frequently occurring reactions reported immediately after surgery include joint swelling, arthralgia and pyrexia. These were generally mild and disappeared in the weeks following surgery.

Adverse reactions reported in patients implanted with ChondroCelect are provided in the table below. The following categories are used to rank the undesirable effects by frequency of occurrence: very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100). Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

System organ class	Very common ≥1/10	Common ≥1/100 to <1/10	Uncommon ≥1/1,000 to <1/100
Psychiatric disorders			Anxiety
Nervous system disorders		Autonomic neuropathy, Complex regional pain syndrome, Pain in extremity, Peripheral neuropathy, Syncope, Trendelenburg's symptom	Hyperesthesia, Migraine, Photophobia, Transient ischaemic attack
Vascular disorders		Deep vein thrombosis, Haematoma, Superficial phlebitis	Fat embolism, Thrombophlebitis
Respiratory, thoracic and mediastinal disorders		Apnoea	Lung embolism
Gastrointestinal disorders		Nausea	
Skin and subcutaneous tissue disorders		Wound infection, Erysipelas, Erythema, Hypertrophic scar, Postoperative wound complication, Pruritus, Scar pain, Wound dehiscence, Wound secretion	Itching scar
Musculoskeletal and connective tissue disorders	Arthralgia, Cartilage hypertrophy, Joint crepitation, Joint swelling	Arthrofibrosis, Joint range of motion decreased, Joint effusion, Joint lock, Arthritis, Arthropathy, Bone cyst, Bone swelling, Bursitis, Chondropathy, Exostosis, Haemarthrosis, Joint instability, Joint stiffness, Loose body in joint, Mobility decreased, Muscle atrophy, Osteoarthritis, Synovial cyst, Synovitis, Tendon disorder, Tendonitis	Chondromalacia, Gonarthrosis

System organ class	Very common ≥1/10	Common ≥1/100 to <1/10	Uncommon ≥1/1,000 to <1/100
General disorders and administration site conditions		Ineffective therapeutic product, Gait disturbance, Impaired healing, Implant site hypersensitivity, Peripheral edema, Pyrexia	Atrophy, Discomfort, Granulomatous lesion
Investigations		Arthroscopy	
Injury, poisoning and procedural complications		Graft complication, Graft delamination, Cartilage injury, Injury, Joint injury, Procedural site reaction	

Adverse reactions of special interest

Arthrofibrosis

In the compassionate use patients, a higher incidence of arthrofibrosis and decreased joint range of motion was observed in a subgroup of patients with a patellar lesion (8.2% and 13.1% respectively) compared to non-patellar lesions (0.6% and 2.6% respectively).

Cartilage hypertrophy

In the majority of the 370 patients included in the Compassionate Use Program, a collagen membrane instead of a periosteal flap was used to seal the defect. According to current literature the incidence of cartilage hypertrophy can be reduced by using a collagen membrane to cover the lesion site instead of using a periosteal flap (Gooding *et al*., 2006; Niemeyer *et al*., 2008). When a collagen membrane was used to seal the lesion site after application of ChondroCelect, the incidence of cartilage hypertrophy was reported to be 1.8% compared to 25% in the randomized, controlled trial alone.

4.9 Overdose

No case of overdose has been reported.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: {group}, ATC code: {code} <not yet assigned>

Conventional pharmacodynamic studies for ChondroCelect have not been performed.

Clinical efficacy

The efficacy of ChondroCelect was studied in a phase III, multicenter, randomized, controlled trial (TIG/ACT/01/2000) and the first two years of its 4-years extension phase (TIG/ACT/01/2000EXT). ChondroCelect was compared to the procedure of microfracture in the repair of symptomatic single cartilage lesions of the femoral condyles of the knee. 51 patients were treated with ChondroCelect, 61 patients were treated with microfracture. Patients aged between 18 and 50 years, who had a single symptomatic cartilage lesion between 1 and 5 cm^2 of the femoral condyles met the inclusion criteria. Patients could be treatment-naïve or might have undergone previous arthroscopic or other surgical repair procedure(s). Patients with patellofemoral cartilage lesion, OCD, depth of lesion >0.5 cm, prior meniscal transplant, prior mosaicplasty and prior microfracture within the last 12 month were

excluded. Patients had to agree to actively participate in a strict rehabilitation protocol and follow-up program.

The median time since onset of knee injury was slightly longer in the ChondroCelect group than in the microfracture group (2.0 years versus 1.6 years). More patients in the ChondroCelect treatment group, compared to patients in the microfracture group, had undergone previous knee surgery (88% versus 77%). In the ChondroCelect group 77% of patients had a medial and 23% a lateral condyle defect.

Histological examination of the repair biopsy at 12 months showed superior structural repair in the ChondroCelect arm compared to the microfracture arm. There was continuous improvement up to 36 months in the clinical outcome measure KOOS (the Knee Injury and Osteoarthritis Outcome Score) in both treatment arms. The estimated benefit was larger in the ChondroCelect group but the results did not reach statistical significance. At this time point 41 patients were evaluated in the ChondroCelect arm and 49 were evaluated in the microfracture arm. Patients with less than 3 years since onset of symptoms (n=27 in the ChondroCelect arm and n=32 in the microfracture arm) benefited most from ChondroCelect. For the group with a longer time since onset of symptoms there were no apparent differences between the 2 groups. Re-intervention on the treated lesion for graft delamination or periost loosening occurred in 2 of 51 patients within 36 months after ChondroCelect implantation, compared to 7 of 61 patients treated with microfracture having generally insufficient or inadequate cartilage repair.

Patients with lesions larger than 5 cm² have been treated under compassionate use only. The safety data obtained in these patients do not indicate a particular safety concern. Further clinical data in patients with larger lesions are foreseen to be collected in the future.

Sixteen patients below 18 years have been treated with ChondroCelect under compassionate use. No specific safety signal was detected in these patients. If, based on the benefit/risk assessment of the responsible surgeon treatment of patients below 18 years is considered, special attention should be given to ensure that the growth plate is completely closed.

5.2 Pharmacokinetic properties

The product is implanted locally.
The nature and intended clinical use of ChondroCelect are such that conventional studies on pharmacokinetics, absorption, distribution, metabolism and elimination are not applicable.

5.3 Preclinical safety data

Non-clinical data based on implantation of expanded cartilage cells in goats and mice did not reveal special hazard for humans.
In studies in goats, mild signs of synovitis were observed in the majority of the animals, including controls at 10 weeks post surgery. Inflammation resolved with time and parameters returned to baseline levels with only some very mild and local signs of synovitis remaining in a few animals. Although it is thought that these reactions are mostly surgery-related, a potential influence of the expanded chondrocytes can not be completely excluded.
In a study in sheep, the majority of animals showed penetration of the transplanted cells in subchondral bone; in two of these cases complete penetration of underlying bone marrow was observed. This finding might be related to the inability to perform a progressive loading under non-weight bearing conditions post-surgery in these models and therefore cannot be fully extrapolated as such to the human situation.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Dulbecco's Modified Eagles Medium (DMEM) (containing amino acids, vitamins, salts and carbohydrates).

6.2 Incompatibilities

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

6.3 Shelf life

48 hours.

6.4 Special precautions for storage

Store between 15°C – 25°C.
Do not refrigerate or freeze.
Keep the product vial(s) within the falcon tube in the outer plastic screw top container in order to protect from light and bacterial/fungal contamination.
Do not irradiate.

6.5 Nature and contents of container and special equipment for use, administration or implantation

ChondroCelect is supplied as one individual treatment dose (falcon tube) contained in 1 to 3 Type I glass vials of 1 ml. Each vial contains 0.4 ml of autologous human cartilage cells suspension and is closed with a chlorobutyl stopper and aluminium seal.
The vials are placed in a sterile falcon tube with a plastic screw top.
The falcon tube is placed in a plastic screw top container together with surgery materials (one sterile syringe of 1 ml, one 18G intravenous catheter and two pieces of Vicryl 6.0) and a temperature monitor.

6.6 Special precautions for disposal and other handling

ChondroCelect is intended solely for autologous use. Prior to implantation match the patient name to the patient/donor identification on the shipment documentation and product vial.

Before administration, ChondroCelect should be resuspended by gently tapping the vial to bring the cells back into suspension.

ChondroCelect should not be sterilised. If the ChondroCelect vial is damaged or its sterility has been compromised, the product must not be used and must be shipped back to TiGenix.

Any unused product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

TiGenix NV
Romeinse straat 12/2
B-3001 LEUVEN
Belgium

8. MARKETING AUTHORISATION NUMBER(S)

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

10. DATE OF REVISION OF THE TEXT

Detailed information on this product is available on the website of the European Medicines Agency (EMEA) http://www.emea.europa.eu

ANNEX II

A. MANUFACTURER OF THE BIOLOGICAL ACTIVE SUBSTANCE AND MANUFACTURING AUTHORISATION HOLDER RESPONSIBLE FOR BATCH RELEASE

B. CONDITIONS OF THE MARKETING AUTHORISATION

A. MANUFACTURER OF THE BIOLOGICAL ACTIVE SUBSTANCE AND MANUFACTURING AUTHORISATION HOLDER RESPONSIBLE FOR BATCH RELEASE

Name and address of the manufacturer(s) of the biological active substance(s)

TiGenix NV
c/o U.Z. Gasthuisberg
Centraal Dienstengebouw, level 2. Herestraat 49
BE-3000 Leuven
Belgium

Name and address of the manufacturer(s) responsible for batch release

TiGenix NV
c/o U.Z. Gasthuisberg
Centraal Dienstengebouw, level 2. Herestraat 49
BE-3000 Leuven
Belgium

B. CONDITIONS OF THE MARKETING AUTHORISATION

- **CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE IMPOSED ON THE MARKETING AUTHORISATION HOLDER**

Medicinal product subject to restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

The Marketing Authorisation Holder (MAH) shall ensure that the medicinal product will be distributed only to Healthcare Establishments that meet criteria described in the Risk Management Plan.

- **CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT**

The Marketing Authorisation Holder (MAH) shall ensure, prior to the distribution of the product to a particular Healthcare Establishment, that all surgeons and other healthcare professionals involved in the handling and administration of ChondroCelect or its components, as well as those involved in follow-up of patients treated with ChondroCelect in the Healthcare Establishment, receive training as per the educational programme described in the Risk Management Plan.

The educational programme for healthcare professionals contains the following components:
- Training material for Surgeons
- Training material for other Healthcare Professionals
- Informed consent for the patients to be signed prior to the treatment with ChondroCelect

The training materials for Surgeons shall include the following key messages and components:
- Summary of Product Characteristics
- The biopsy harvest procedure
- The surgical checklist to be completed at the operating theatre immediately prior to the first incision confirming the right patient, the right product, the right side of the implantation, and the type of biological membrane and fibrin sealant to be used in the procedure.
- The implantation procedure by knee-joint arthrotomy
- The follow-up protocol

The training material for other Healthcare Professionals shall include the following key messages and components:

- Summary of Product Characteristics
- The need for screening of donors using patient questionnaire and laboratory tests for hepatitis C, hepatitis B, HIV, and Syphilis
- The handling of the biopsy harvest
- The handling of ChondroCelect and its preparation for the implantation
- The schedule of follow-up of patients
- The recommended physiotherapy

- **OTHER CONDITIONS**

Pharmacovigilance system
The MAH must ensure that the system of pharmacovigilance, as described in version 1 dated (03/05/2007) presented in Module 1.8.1. of the Marketing Authorisation Application, is in place and functioning before and whilst the product is on the market.

Risk Management Plan
The MAH commits to performing the studies and additional activities detailed in the Pharmacovigilance Plan and in the Efficacy Follow-up plan, as agreed in version 4 (dated 22/06/2009) of the Risk Management Plan (RMP) presented in Module 1.8.2. of the Marketing Authorisation Application and any subsequent updates of the RMP agreed by the CAT and the CHMP.

As per the Volume 9A of the Rules Governing Medicinal Products in the EU, the updated RMP should be submitted at the same time as Periodic Safety Update Reports (PSURs).

In addition, an updated RMP should be submitted

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan, Efficacy Follow-up Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance, efficacy, or risk minimisation) milestone being reached
- At the request of the EMEA

ANNEX III

LABELLING AND PACKAGE LEAFLET

A. LABELLING

PARTICULARS TO APPEAR ON THE OUTER PACKAGING

White screw top container

1. NAME OF THE MEDICINAL PRODUCT

ChondroCelect 10,000 cells/microlitre implantation suspension.

Characterised viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins.

2. STATEMENT OF ACTIVE SUBSTANCE(S)

Each vial contains 4 million autologous human cartilage cells in 0.4 ml, corresponding to a concentration of 10,000 cells/microlitre.

3. LIST OF EXCIPIENTS

Dulbecco's Modified Eagles Medium (DMEM).

4. PHARMACEUTICAL FORM AND CONTENTS

Implantation suspension.
1 falcon tube with 1, 2 or 3 vials (x 0.4 ml)
Vials are supplied with surgery materials (one sterile syringe of 1 ml, one 18G IV catheter and two pieces of Vicryl 6.0 sutures)

5. METHOD AND ROUTE(S) OF ADMINISTRATION

For implantation.
Read the package leaflet before use.

6. SPECIAL WARNING THAT THE MEDICINAL PRODUCT MUST BE STORED OUT OF THE REACH AND SIGHT OF CHILDREN

Keep out of the reach and sight of children.

7. OTHER SPECIAL WARNING(S), IF NECESSARY

For autologous use only.

8. EXPIRY DATE

EXP {DD month YYYY} at {hours} CET

9. SPECIAL STORAGE CONDITIONS

Store between 15°C – 25°C.
Do not refrigerate or freeze.
Keep the product vial(s) within the falcon tube in the outer plastic screw top container in order to protect from light and bacterial/fungal contamination.
Do not expose to radioactive irradiation (X-rays).

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Dispose of in accordance with local requirements.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

TiGenix nv, Romeinse straat 12/2, B-3001 Leuven, Belgium
Tel: +32-(0)16 39 60 60
Fax: +32-(0)16 39 60 70
info@tigenix.com

12. MARKETING AUTHORISATION NUMBER(S)

EU/0/00/000/000

13. BATCH NUMBER, DONATION AND PRODUCT CODES

Lot {lot number}
Patient number (Pt N°) {patient number}
Patient Initials (Pt initials) {patient intitials}

14. GENERAL CLASSIFICATION FOR SUPPLY

Medicinal product subject to medical prescription.

15. INSTRUCTIONS ON USE

16. INFORMATION IN BRAILLE

Justification for not including Braille accepted

MINIMUM PARTICULARS TO APPEAR ON SMALL INTERMEDIATE PACKAGING UNITS

Falcon tube

1. NAME OF THE MEDICINAL PRODUCT

ChondroCelect 10,000 cells/microlitre implantation suspension.

2. METHOD OF ADMINISTRATION

3. EXPIRY DATE

EXP {DD month YYYY} at {hours} CET

4. BATCH NUMBER, DONATION AND PRODUCT CODES

Lot {lot number}
Pt N° {patient number}
Pt Initials {patient intitials}

5. CONTENTS BY WEIGHT, BY VOLUME OR BY UNIT

1, 2 or 3 vials x 0.4 ml

6. OTHER

For autologous use only.

MINIMUM PARTICULARS TO APPEAR ON SMALL IMMEDIATE PACKAGING UNITS

Vial

1. NAME OF THE MEDICINAL PRODUCT

ChondroCelect

2. METHOD OF ADMINISTRATION

3. EXPIRY DATE

EXP {DD month YYYY} at {hours} CET

4. BATCH NUMBER, DONATION AND PRODUCT CODES

Lot {lot number}
Pt N° {patient number}
Pt Initials {patient intitials}

5. CONTENTS BY WEIGHT, BY VOLUME OR BY UNIT

0.4 ml

5. OTHER

For autologous use only.

B. PACKAGE LEAFLET

PACKAGE LEAFLET: INFORMATION FOR THE USER

ChondroCelect 10,000 cells/microlitre implantation suspension
Characterised viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins

Read all of this leaflet carefully before you start using this medicine.
- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor, surgeon or physical therapist.
- If any of the side effects gets serious, or if you notice any side effects not listed in this leaflet, please tell your doctor, surgeon or physical therapist.

In this leaflet:

1. WHAT CHONDROCELECT IS AND WHAT IT IS USED FOR

ChondroCelect consists of autologous cultured cartilage cells. The product is made from a small sample of cartilage cells (a biopsy) taken from your knee.

- **Autologous** means that your own cells are used to make ChondroCelect.

- **Cartilage** is a tissue that is present in every joint. It protects the ends of our bones and allows our joints to function smoothly.

ChondroCelect is used to repair single symptomatic cartilage defects in the femoral condyle of the knee in adults. A defect can be caused by acute trauma, such as a fall. It can also be caused by repetitive trauma, such as long-term incorrect weight-bearing on the knee.

- The **femoral condyle** is the end of the thigh bone, which forms part of your knee.

2. BEFORE YOU USE CHONDROCELECT

Do not use ChondroCelect
If you are allergic (hypersensitive) to any of the ingredients of ChondroCelect or to bovine serum
If you suffer from advanced osteoarthritis (degenerative joint disease) in your knee.

Take special care with ChondroCelect

If you have an acute or recent history of bone or joint infections, you should be temporary deferred until documented recovery.

The use of ChondroCelect is not recommended when you have overweight (i.e. a Body Mass Index over 30). Your surgeon will give you more information.

ChondroCelect is not recommended for the repair of cartilage defects in other locations than the femoral condyle.

The use of ChondroCelect is not recommended in children and adolescents below 18 years.
Limited data are available on adult patients older than 50 years.

ChondroCelect should be implanted in an otherwise healthy knee. This means that other knee problems such as lesions of the knee ligament or of the meniscus should be corrected before or during ChondroCelect implantation.

You should resume physical activity according to the rehabilitation plan recommended by the physical therapist. Too early and vigorous activity may compromise the implant and the durability of clinical benefit from ChondroCelect.

Your surgeon will give you more information on any special considerations for your particular case.

Other cases in which ChondroCelect cannot be supplied
Even if the surgeon has already taken a small sample of cartilage cells (a biopsy) needed to produce the product, it is possible that you will not be eligible for treatment with ChondroCelect. This is the case if the biopsy is of insufficient quality to make ChondroCelect, or in some instances, it may be the cells cannot be grown in the laboratory or that the expanded cells do not meet all the quality requirements Your surgeon will be informed and might have to select an alternative treatment for you.

Using other medicines
The safe use of ChondroCelect with other medicines has not been studied.
Ask your doctor for more information as to which pain medication you can safely use.
Please tell your doctor or physical therapist if you are taking or have recently taken any other medicines, including medicines obtained without a prescription.

Pregnancy and breast-feeding
The safe use of ChondroCelect has not been demonstrated during pregnancy or breast-feeding.
ChondroCelect is not recommended for pregnant and breast-feeding women.
Please inform your doctor if you are pregnant or think you may be pregnant.

Driving and using machines
The surgical procedure will have a major influence on your ability to drive and use machines. Driving cars and using machines may be limited during the rehabilitation period, and the advice of your doctor, surgeon or physical therapist should be strictly followed during this period.

3. HOW TO USE CHONDROCELECT

ChondroCelect can only be prescribed and implanted by an orthopaedic surgeon in a hospital.
Treatment with ChondroCelect: a two-step procedure

Visit 1: evaluation of the cartilage defect and biopsy
On the first visit, the surgeon will evaluate your cartilage defect during an exploratory operation (arthroscopy). An arthroscopy is performed through very small incisions in the skin, using a narrow telescope (arthroscope) to look at the inside of the knee. If the surgeon decides that treatment with ChondroCelect is appropriate for you, he/she will take a small sample of cartilage cells (a biopsy) from your knee. This cartilage sample will be used to make ChondroCelect.
It will take at least four weeks to select and culture the cells to make ChondroCelect.

Visit 2: ChondroCelect implantation
During open-knee surgery, the cartilage cells are implanted into the cartilage defect. This is called 'autologous chondrocyte implantation' (ACI). The purpose is to repair the defect with healthy and functional cartilage over time.
To keep the cartilage cells in place, a biological membrane is sewn over the defect.

Rehabilitation
After surgery, you will have to follow a rehabilitation program for approximately one year, to allow your knee to heal well. Your doctor or physical therapist will give you more details on your rehabilitation.

It is very important to carefully observe the recommendations of your doctor and/or physical therapist. If you do not follow your rehabilitation schedule, the risk of treatment failure may increase.

You should be very cautious when bending and putting weight on your treated knee. During the rehabilitation period, the level of weight-bearing will increase gradually, depending on your weight and the size of the cartilage defect. To protect your knee, you will have to wear a brace.

Ask your doctor or physical therapist if you have any further questions about the treatment with ChondroCelect.

4. POSSIBLE SIDE EFFECTS

Like all medicines, ChondroCelect can cause side effects, although not everybody gets them.

Most side effects of ChondroCelect implantation are side effects related to open-knee surgery. In general, these side effects are quite mild and disappear in the weeks following surgery.

You can recognize most of the joint- related side effects if you have symptoms like pain, snapping, grinding, locking, swelling, bending limitations and stiffness in the knee. Tell your doctor immediately if you notice any of these symptoms.

The frequency of possible side effects listed below is defined using the following convention:
- very common (affects more than 1 user in 10)
- common (affects 1 to 10 users in 100)
- uncommon (affects 1 to 10 users in 1,000)
- rare (affects 1 to 10 users in 10,000)
- very rare (affects less than 1 user in 10,000)
- not known (frequency cannot be estimated from the available data)

Very common side effects (likely to occur in more than 1 in 10 patients) include: joint pain (arthralgia), overgrowth of cartilage cells (cartilage hypertrophy), crackling or clicking sensation when articulating the knee (joint crepitation), and joint swelling.

Common side effects (likely to occur in 1 to 10 patients in 100) include: restriction of knee montion (arthrofibrosis, decreased joint range of motion, decreased mobility), excessive amount of joint fluid in the joint (joint effusion), joint lock, joint inflammation (arthritis, bursitis, synovitis), cavity filled with fluid in the knee (bone cyst, synovial cyst), bone swelling, cartilage disorder (chondropathy), benign bony growth (exostosis), blood in a joint (haemarthrosis), joint instability, joint stiffness, loose body in joint, weakening of muscle (muscle atrophy, Trendelenburg's sign), degenerative joint disorder (osteoarthritis), tendon disorder, inflammation of the tendon (tendonitis), impaired healing, treatment failure, gait disturbance, implant site hypersensitivity, peripheral edema, fever (pyrexia), postoperative wound complication (wound site reaction), loosening of the graft or membrane (graft complication, graft delamination), injury (cartilage injury, joint injury), blood cloth in the deep vein of the leg (deep

vein thrombosis), large bruise (haematoma), superficial vein inflammation (phlebitis), nausea, pain or nerve disorder (peripheral neuropathy, complex regional pain syndrome, autonomic neuropathy), syncope, apnea, arthroscopy.

Uncommon side effects (likely to occur in 1 to 10 patients in 1,000) include: anxiety, hypersensitivity (hyperesthesia, photophobia), migraine, mini stroke (transient ischaemic attack), fat entering the circulatory system (fat embolism), vein inflammation (thrombophlebitis), blockage in a lung artery (lung embolism), itching scar, pain at the front of the knee (chondromalacia), breakdown of tissue (gonartrhosis, atrophy), discomfort, chronic inflammation (granulomatous lesion).

Long-term experience with the implantation of cartilage cells is limited. Therefore, it is possible that complications or side effects as yet unknown may occur.

If any of the side effects gets serious, or if you notice any side effects not listed in this leaflet, please contact your doctor or physical therapist.

5. HOW TO STORE CHONDROCELECT

Keep out of the reach and sight of children.

Do not use ChondroCelect after the expiry date which is stated on the container and vial after EXP.

Store between 15°C – 25°C.
Do not refrigerate or freeze.
Keep the product vial(s) within the falcon tube in the plastic screw top container in order to protect from light and bacterial/fungal contamination.
Do not irradiate.

Since this product will be used during your knee surgery, the hospital staff is responsible for the correct storage of the product both before and during its use, as well as for the correct disposal

6. FURTHER INFORMATION

What ChondroCelect contains
The active substance of ChondroCelect consists of a treatment dose of viable autologous human cartilage cells in vials containing 4 million cells in 0.4 ml, corresponding to a concentration of 10,000 cells/microlitre.
The other ingredient is sterile, buffered Dulbecco's Modified Eagles Medium (DMEM), a liquid containing amino acids, vitamins, salts and carbohydrates to store the cells in the vial.

What ChondroCelect looks like and contents of the pack
ChondroCelect is a cell suspension (a fluid) for implantation. The cells are kept alive in a small sterile vial. The product is packaged in several layers of packaging materials which guarantee sterility and stable temperature conditions for 48 hours if stored at room temperature.
Each packaging contains an individual treatment dose consisting of 1 to 3 vials, depending on the number of cells needed to treat the specific lesion size.

Marketing Authorisation Holder and Manufacturer

TiGenix nv
Romeinse straat 12/2, 3001 LEUVEN
Belgium
+32 16 39 60 60
+32 16 39 60 70
info@tigenix.com

The leaflet was approved in

Detailed information on this medicine is available on the European Medicines Agency (EMEA) web site: http://www.emea.europa.eu.

European Medicines Agency
Evaluation of Medicines for Human Use

EMEA/724428/2009

ASSESSMENT REPORT

FOR

ChondroCelect

Common name: characterised viable autologous cartilage cells expanded ex vivo expressing specific marker proteins

Procedure No. EMEA/H/C/000878

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 16
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

TABLE OF CONTENTS

1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The Applicant TiGenix NV submitted on 01 June 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for ChondroCelect, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMEA/CHMP on 26 September 2006.

The legal basis for this application refers to:

A - Centralised / Article 8(3) / New active substance.

Scientific Advice:
The Applicant received Scientific Advice from the CHMP on 28 April 2008 (EMEA/151996/2206). The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status:
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:
Rapporteur: **Christian K. Schneider** Co-Rapporteur: **Jaana Kallio**

As ChondroCelect is an Advanced Therapy medicinal product, the advanced therapy regulation was applicable to this procedure. Therefore, during the CHMP meeting of 12 – 13 February 2009, a CAT Rapporteur, a CAT Co-Rapporteur and a CHMP Co-ordinator were appointed.

Rapporteur: **Egbert Flory** Co-Rapporteur: **Paula Salmikangas**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 01 June 2007.
- The procedure started on 20 June 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 03 September 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 03 September 2007. In accordance with Article 6(3) of Regulation (RC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- During the meeting on 18 October 2007, the CHMP agreed on the consolidated List of Questions to be sent to the Applicant. The final consolidated List of Questions was sent to the Applicant on 18 October 2007.
- The Applicant submitted the responses to the CHMP consolidated List of Questions on 28 April 2008.
- The final report of inspections carried out at the manufacturing site in Belgium on 13-14 December 2007 and 20-21 May 2008 was issued on 17 June 2008.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant’s responses to the List of Questions to all CHMP members on 09 June 2008.
- During the CHMP meeting on 26 June 2008, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the Applicant.
- The Applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 03 September 2008.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant’s responses to the List of Outstanding Issues to all CHMP members on 11 September 2008.

- During a meeting of an Ad Hoc Expert group / Biologics Working Party on 13 October 2008, experts were convened to address questions raised by the CHMP.
- During the CHMP meeting on 23 October 2008, outstanding issues were addressed by the Applicant during an oral explanation before the CHMP. The CHMP agreed on a second list of outstanding issues to be addressed in writing and in an oral explanation by the Applicant.
- During the CHMP meeting of 12 – 13 February 2009 Dr. Egbert Flory was appointed as CAT Rapporteur and Dr Paula Salmikangas was appointed as CAT CoRapporteur.
- The Applicant submitted the responses to the CHMP consolidated second List of Outstanding Issues on 24 April 2009.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the 2nd List of Outstanding Issues to all CHMP and CAT members on 11 May 2009.
- During the CAT meeting on 14 May 2009, outstanding issues were addressed by the Applicant during an oral explanation before the CAT.
- During the CAT meeting on 14 May 2009, a 3rd List of Outstanding Issues was adopted by CAT. The CHMP endorsed the 3rd LoOI on 29 May 2009.
- The Applicant submitted the responses to the third List of Outstanding Issues on 03 June 2009.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant's responses to the 3rd List of Outstanding Issues to all CHMP and CAT members on 12 June 2009.
- The Applicant provided the letter of undertaking on follow-up measures to be fulfilled post-authorisation on 23 June 2009.
- On 24 June 2009, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive draft opinion for granting a Marketing Authorisation to ChondroCelect by written procedure including the recommendation under Article 14(2) of Regulation (EC) No 1394/2007 that the Marketing Authorisation Holder performs the studies and additional activities detailed in the Pharmacovigilance Plan and in the Efficacy Follow-up plan, as agreed in version 4 (dated 22/06/2009) of the Risk Management Plan (RMP) presented in Module 1.8.2. of the Marketing Authorisation Application and any subsequent updates of the RMP agreed by the CAT.
- During the meeting on 25 June 2009, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to ChondroCelect including the recommendation under Article 14(2) of Regulation (EC) No 1394/2007 that the Marketing Authorisation Holder performs the studies and additional activities detailed in the Pharmacovigilance Plan and in the Efficacy Follow-up plan, as agreed in version 4 (dated 22/06/2009) of the Risk Management Plan (RMP) presented in Module 1.8.2. of the Marketing Authorisation Application and any subsequent updates of the RMP agreed by the CHMP.

2. SCIENTIFIC DISCUSSION

2.1 Introduction

Joint surface defects can originate after trauma, after osteochondritis dissecans or can be caused by an underlying genetic predisposition. The healing capacity of articular cartilage is poor and damaged articular cartilage is thought to be a precursor to the development of osteoarthritis. Damaged articular cartilage can result in pain, loss of joint function and disability. An early intervention on symptomatic cartilage lesions may prevent or delay irreversible changes in the joint surface.

Currently, there is no uniform approach to managing significant knee cartilage defects. Interventions that aim to provide symptomatic relief include debridement, lavage and rehabilitation. Interventions intended to re-establish the cartilage surface include marrow stimulation techniques (i.e. microfracture (MF), abrasion arthroplasty or drilling), mosaicplasty and autologous chondrocyte implantation (ACI). Microfracture is frequently used as treatment for patients with smaller articular cartilage defects of the knee (for lesions < $4cm^2$). It induces cartilage repair by penetrating the subchondral bone and stimulating bleeding and thus the formation of a fibrin clot, which is considered to stimulate fibro cartilage formation, and has been shown to result in functional improvements within the first 2 years following treatment. For larger lesions particularly those exceeding $4cm^2$, however, this procedure is not recommended.

Mosaicplasty takes advantage of the limited self-renewal capacity of the joint surface by fitting one or several osteochondral plugs, obtained from a low weight bearing area of the joint into a mosaic. It transforms large defects into several small defects that can be repaired spontaneously by the surrounding tissue and by the invading bone marrow derived skeletal precursors/mesenchymal stem cells.

The ACI procedure was first developed in 1994 described by Brittberg et *al*. (1994) using a first generation autologous chondrocyte product. In the following years many groups could demonstrate the benefit and formation of 'cartilage repair tissue' with long-lasting stability and symptomatic relief between two and nine years after ACI treatment. For larger lesion sizes exceeding $4cm^2$, ACI is also considered a suitable treatment option.

ChondroCelect by Tigenix nv is a medicinal product for use in ACI treatment. ChondroCelect is a suspension of approximately 10,000 cartilage cells per microliter of medium for autologous use. The cells have been obtained by ex vivo expansion of chondrocytes isolated from a biopsy of the articular cartilage from the patient's knee.

Treatment with ChondroCelect comprises a two-step surgical procedure. In the first step a cartilage biopsy is obtained arthroscopically from healthy articular cartilage from a lesser weight bearing area of the patient's knee, approximately 4 weeks prior to implantation. Chondrocytes are isolated from the biopsy by enzymatic digestion, expanded *in vitro*, characterised and delivered as a suspension of 1 x 10^4 cells/µl for implantation in the same patient. During the second step of the procedure the expanded chondrocyte suspension is implanted in an open-knee surgery. In the pivotal study a periosteal flap was harvested from the medial tibia, sutured into the defect, with the cambium layer facing the subchondral bone, and sealed with fibrin glue. In future applications the defect will be covered with the help of a biodegradable membrane. The dosage of the cell suspension is defined as 0.8 to 1 million cells per cm^2 defect size. Hence, depending on the defect size measured at biopsy procurement, 4 or 8 or 12 million cells are formulated into 1 or 2 or 3 vial(s) of 4 million cells/ 0.4 ml excipient.

The claimed indication for ChondroCelect is repair of single symptomatic cartilaginous defects of the femoral condyle of the knee (ICRS grade III or IV) in adults.

2.2 Quality aspects

Introduction

ChondroCelect is an autologous cell-based medicinal product consisting of chondrocytes that were expanded ex vivo after sourcing from a small biopsy of healthy cartilage from a lesser weight bearing area of the same patient's damaged knee.

The active substance consists of autologous cartilage forming (chondrogenic) cells which are characterised by specific marker proteins.

For details on the composition of ChondroCelect please refer to Table 1.

Table 1. Composition of ChondroCelect

Substance	Function	Content
Pellet of washed cells	Active Substance	4 Mio Cells/ 0,4 ml
Dulbecco's Modified Eagle Medium with glucose, without phenol red	Excipient	0,4 ml

Active Substance

The Active Substance is a centrifuged pellet of 4 to 12 million cells that were expanded *ex vivo,* harvested and washed. The expansion process is designed to preserve the integrity and function of the cells and particularly to maintain the cells' ability to produce hyaline cartilage. This method has been developed and validated in order to limit the usually observed dedifferentiation of chondrocytes in culture. Lineage marker analysis are performed in order to demonstrate that the culture conditions do not enrich for other cell lineage populations for example fibroblasts and provide reassurance of the homogeneity of the ChondroCelect cell population.

- Manufacture

Biopsy procurement

The starting material consists of an autologous articular cartilage biopsy procured arthroscopically from a non weight-bearing area of the femoral condyle of the patient's knee. The Applicant provides hospitals with biopsy procurement kits, which are stored at the orthopaedic unit. Each kit is labelled with a unique lot number on the outer box and the containers within.

Eligible patients for ChondroCelect treatment are screened for HIV type 1 and 2, HCV, HBV, and syphilis. Only tissue from donors who test negative will be released from quarantine and allowed into the tissue/cell processing area.

The orthopaedic surgeon will plan an arthroscopy to assess the cartilage lesion and procure a cartilage tissue biopsy. The cartilage tissue biopsy is aseptically transferred into sterile biopsy medium.

The biopsy kit is conditioned prior to shipment and is transported under strict monitoring of the temperature during transport. Upon receipt of the biopsy kit, the biopsy is quarantined until successful donor screening results are available.

Each biopsy kit is identified with a unique lot number which is composed of the following elements: the date on which the biopsy kit is assembled, the product type and the batch sequence number. At the time of arthroscopy the surgeon records two patient identifiers, i.e. donor initials and the patient's administration number in the hospital (hospital identifier) on the patient form and thus links the patient

identifiers to the lot number of the biopsy kit. These three codes are designed to form a unique combination, and the biopsy lot number is used throughout the entire process to identify the donor's tissue and all lot related materials and documentation.

Manufacturing process

The manufacturing process of the Active Substance consists of the following steps:

- Biopsy digestion
- Expansion culture
- Cell culture harvest and wash

Biopsy digestion

Before further processing, the appearance of the biopsy medium is verified with respect to clarity and colour. The tissue is minced under aseptic conditions and bone fragments are removed. The cartilage fragments are then transferred to allow for dissociation of the cartilage tissue fragments and to release the chondrocytes from the tissue matrix. Chondrocytes are isolated, washed, counted and seeded in tissue culture flasks in culture medium.

Expansion culture

The isolated cells are transferred to an incubator with humidified atmosphere and replenished with fresh culture medium in regular intervals. The flasks are regularly inspected. When the cultures reach confluence the cells are dissociated from the flask surface and subcultured in fresh tissue culture flasks until the appropriate number of expanded cells has been reached.

The spent medium is pooled from every flask and sampled for microbiological testing

The total number of passage numbers should remain lower or equal than 3

Cell culture harvest and wash

At the end of culture the cells are trypsinized and collected, centrifuged and washed thoroughly. Cell viability is verified and a gram stain is performed on the collected wash solution. The cells obtained as a pellet at this stage are considered the Active Substance (i.e. living human autologous cartilage forming cells).

In-process controls and specifications

The in-process controls of the manufacturing process have been clearly specified.
Critical parameters have been included as in process controls to routinely confirm the quality of the Medicinal product by testing the biopsy and cell culture for aspects like e.g. medium appearance, pH, microbiology, cell morphology, purity, cell viability and cell yield. Appropriate operating ranges have been defined. As the manufacturing process is a continuous process and the active substance is not stored in between, no formal Active Substance specifications have been set.

Process validation and characterisation of Active Substance

The Applicant used a series of functional tests capable to characterise the cells and suitable to validate the manufacturing process. These functional assays include a cell culture (3D cell culture assay), an *in vitro* assay in animal models and cellular expression patterns of genes relevant for cartilage and chondrocyte biology.

The validation of the manufacturing process has been adequately performed. Some minor issues on the acceptance criteria for some parameters followed during the validation are still outstanding. The Applicant has committed to explore further the specification limits for the functional assay and to

further define the acceptance criteria for process validation. On basis of the validation approach, the data collected and the commitment on further exploration of the specification limits, the comparability and consistency of lots produced by the proposed manufacturing process have been demonstrated.

Medicinal product

The manufacturing process from the Active Substance to the Medicinal product is a continuous process without intermediate holding steps. The cell pellet is immediately resuspended in the excipient nutrient medium and packaged for shipment. The dosage is defined as 0.8 to 1 million cells per cm^2 defect size. Hence, depending on the defect size measured at biopsy procurement, 4 or 8 or 12 million cells are formulated into 1 or 2 or 3 vial(s) of 4 million cells/ 0.4 ml excipient/ vial. Concentration of the Medicinal product is 10,000 cells formulated per microliter excipient.

- Pharmaceutical development

The Manufacture of ChondroCelect Medicinal product involves the formulation of the cell pellet in the excipient medium and subsequent filling into glass vials. The Applicant has conducted studies to demonstrate the suitability of the transport medium to serve as the excipient of the Medicinal product. The Medicinal product is composed of the Active Substance (a pellet of washed cells) and an aqueous nutrient medium (Dulbecco's Modified Eagle Medium with glucose).

- Manufacture of the product

The Medicinal product is manufactured, routinely controlled and batch released by Tigenix (Leuven, Belgium). Operations are in compliance with Good Manufacturing Practice (GMP).

The re-suspension of the Active Substance, the cell pellet in medium, without any intermediate holding steps yields the Medicinal product. The product is filled into a 1 ml clear, V-shaped, type 1 borosilicate glass vial, which is closed with a grey chlorobutyl /45 stopper. Vial and cap are manually crimp sealed with an aluminium tear-off seal. The glass vial complies with Ph.Eur. requirements. The chlorobutyl stopper is made of material that has "low extractables" characteristics and is certified by the manufacturer to be compliant with all applicable procedures and specifications.

Based on the cell counts from the final harvest, the Medicinal product is formulated to contain 10,000 cells /µl and 0.4 ml of the cell suspension is filled per vial. Each vial thus contains a total of 4 million cells. Depending on the total amount of cells needed to treat a specific lesion, up to three vials are filled and provided within the falcon flask. Since the dosage is 0.8 to 1 million cells per cm^2, the defective area, which may be treated with ChondroCelect, is limited to 15 cm^2.

- Adventitious agents

The raw materials of biological origin used in the production of ChondroCelect include collagenase, fetal bovine serum and porcine trypsin. All raw materials, which are sourced directly or indirectly from animal material, are subjected to a risk analysis procedure and a compliance check with the appropriate legislative requirements. Certificate of suitability from the European Directorate for Quality of Medicinal Products (EDQM) have been provided.

A valid EDQM Certificate of Suitability for foetal bovine serum (FBS) has been provided. Absence from bovine viruses according to Ph. Eur. 01/2008/2262, monograph Serum bovinum and EMEA Guideline (CPMP/BWP/1793/02) has been demonstrated. In addition, test methods have been described in details and virus inactivation results using gamma irradiation have been provided.

Overall, sufficient data is provided to exclude a risk of TSE transmission through ChondroCelect. The risk of transmitting TSE by ChondroCelect is thus considered very remote.

- Product specification

The quality control program performed on the Medicinal product for ChondroCelect includes a test for sterility by Ph. Eur., testing for absence of Mycoplasma by Ph. Eur., testing for absence of endotoxin by Ph. Eur. and gram staining. Dosage and cell viability are confirmed prior to release. Visual tests for absence of particles and vial integrity are performed.

All analytical methods are performed according to Ph. Eur. where applicable and are validated according to ICH guidelines.

Compliance with the product specifications has been demonstrated, and the provided data is considered acceptable. The Company has committed to provide additional data in support of the product specification post-marketing.

- Stability of the product

Stability was addressed by analyzing various lots at time point 0 and time point 48 h. The data reveal no major changes. Hence, a shelf life of 48h is justified for ChondroCelect.

- GMO

ChondroCelect is composed of non-modified human autologous cells. The cells administered to the patient are likely to remain in the implantation site and are not released to the environment. Furthermore, incidental cell leakage is expected to result in metabolism, as is the case for natural release of cells within the body. Therefore the use of ChondroCelect is unlikely to result in any risk to the environment, due to its nature and also because the product is not released into the environment.

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of ChondroCelect Active Substance and Finished product have been presented in a satisfactory manner. The results of the tests carried out indicate satisfactory consistency and uniformity of the manufacturing process and finished product, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

At the time of CAT opinion, there were a number of minor unresolved quality issues related to the specification limits, biodegradable membrane and storage conditions (see above). These shortcomings, however, have no impact on the Risk-benefit balance of the product. The Applicant provided a Letter of Undertaking and committed to resolve these as follow-up measures after the opinion, within an agreed timeframe.

2.3 Non-clinical aspects

Introduction

Non-clinical studies were performed as combined pharmacodynamic / pharmacokinetic (distribution) / toxicological studies in the ectopic mouse (nu/nu model) and in orthotopic models in sheep and goats.

These studies were non-GLP which is not in conformity with the pharmaceutical standards. However, these deficiencies were considered by CHMP to be tolerable in view of the specificity of the development programme for this particular product. In addition human data were supported by adequate clinical studies and did not raise any safety concerns.

Pharmacology

- Primary pharmacodynamics

Nude mouse model

Nude mice received an intramuscular injection of human articular chondrocytes expanded according to the ChondroCelect culture process. Implants retrieved from nude mice at 2 weeks post injection were subjected to histological staining. Based on these characteristics, the cartilage implants were considered of hyaline-like nature. Compared to normal adult human articular cartilage, the cartilage implants were hypercellular and lacked the typical columnar organisation.

A further mouse study was performed using early or late passage expanded human articular chondrocytes. When injected intramuscularly into nude mice, late passage expanded cells did not form any cartilage tissue. In contrast, early passage expanded human articular chondrocytes formed a cartilage implant.

Studies in a large animal species

The importance of phenotypic stability for inducing *in vivo* hyaline-like cartilage formation was investigated by comparing goat or human articular chondrocytes of different passages. The histological score of implants indicated a loss of stable cartilage-forming potential at higher passages.

In goats ChondroCelect-like chondrocytes showed a more stable cartilage forming potential than dedifferentiated chondrocytes. Implantation of ChondroCelect-like autologous chondrocytes resulted in an improved repair efficacy compared to dedifferentiated chondrocytes or dermal fibroblasts as observed in an improved repair in the defect centre, and improved repair tissue integration. Dedifferentiated autologous chondrocytes induced moderate defect filling with poor repair tissue and no or minimal basal and/or lateral integration. In all animals, partial or complete delamination of periosteal flap and fissures in the grafted area and surrounding cartilage was observed.

The repair of the cartilage defect was evaluated by the Modified O'Driscoll (MOD) scores that represent a scoring system to assess late-stage cartilage regeneration. The number of data points was very limited and the MOD score obtained in goats have shown a non-valid correlation with the histology score obtained for the same cell preparations in nude mice.

As observed for goat chondrocytes, the *in vivo* cartilage-forming capacity of human articular chondrocytes in nude mice was progressively lost during *in vitro* cell culture from passage 2-3 onwards. Chondrocytes expanded to higher passage numbers did not form an implant when injected into the thigh of nude mice. In another study in goats comparing passage 1 versus passage 5 expanded human articular chondrocytes according to ChondroCelect culture process all animals showed poor repair, possibly due to an immunological reaction to the human cells.

In a goat study ChondroCelect-like autologous chondrocytes controls were performed with or without periosteal flap. Goats sacrificed up to 53 weeks post-implantation of ChondroCelect-like autologous chondrocytes showed normal mobility, almost complete filling of the cartilage lesion with hyaline-like cartilage or hyaline-like cartilage/fibrocartilage. However, goats sacrificed at various weeks post-implantation showed some degree of bone front ingrowths into the defect. The degree of bone front ingrowths into the defect at 52 weeks was most prominent in animals implanted with ChondroCelect-like cells.

- Secondary pharmacodynamics

The potential formation of other tissue types as a consequence of the loss of phenotypic stability during the *in vitro* expansion process was investigated. Further secondary pharmacodynamic studies were not performed.

- Safety pharmacology programme

ChondroCelect is administered locally. No direct effect of the cells or an effect of secreted pharmacologically active substances on CNS, cardiac or respiratory system is considered for this cell therapy medicinal product, thus the omission of safety pharmacological studies is in line with the guideline on human cell based medicinal products (EMEA/CHMP/410869/2006).

- Pharmacodynamic drug interactions

No formal pharmacodynamic drug interaction studies have been performed, since the Applicant justified that the intended clinical use and the applied surgical procedures are not associated with potential concerns regarding pharmacodynamic interactions with pre-, peri- or post-operatively administered medicinal products.

Fibrin sealants are broadly employed in orthopaedic surgery as an adjunct to haemostasis during total knee prosthesis replacement or as mechanical seal of the outside margins of the membrane used to cover the defect in ACI. Fibrin sealant products differ significantly in their quantitative and qualitative composition, of the active substance and the excipients, thus it cannot be excluded that certain fibrin glues have, due to their composition, a negative effect on the viable cells and/or membrane.

Compatibility data for the fibrin glue TissuCol (Tisseel) have demonstrated the safe and effective use of this sealant with ChondroCelect in non-clinical studies. No interaction studies with any other type of fibrin glues were performed. However the concomitant use of Quixil in the pivotal clinical trial did not reveal any safety signal so far.

Pharmacokinetics

Two studies were performed in goats to evaluate the persistence of cells in the inflicted cartilage defect as well as the potential migration of cells outside the implantation site. These studies with fluorescently-tagged ChondroCelect-like autologous chondrocytes demonstrated that implanted cells become a structural part of newly formed cartilage.

No *pharmacokinetic drug interaction studies* were performed. This is in line with the draft guideline on human cell based medicinal products (EMEA/CHMP/410869/2006).

Toxicology

- Single dose toxicity

Female NMRI nu/nu mice received intramuscular or subcutaneous injections of human articular chondrocytes (freshly isolated or expanded according to the ChondroCelect culture process), goat articular chondrocytes, pig articular or epiphysial chondrocytes, a combination of pig articular chondrocytes and human periosteal cells, goat dermal fibroblasts, immortalized cell lines with chondrocyte characteristics or human synovial membrane-derived mesenchymal stem cells. Two deaths unlikely to be caused by ChondroCelect were observed, all other animals treated were normal and healthy during the course of the experiment, regardless of the number and type of cells administered.

In a sheep study, 70% if the animals receiving an implantation of either autologous articular chondrocytes expanded according to the ChondroCelect culture process, freshly isolated allogeneic articular chondrocytes, freshly isolated human articular chondrocytes or freshly isolated human stem cells showed penetration of cells in subchondral bone, partly with granulomatous reaction. Two of these animals also showed complete penetration of underlying bone marrow.

In goats either autologous cells or human articular chondrocytes, expanded according to the ChondroCelect culture process were implancted via an ACI procedure. In one study an extensive set of safety parameters was monitored. Clinical and laboratory signs observed occurred with low incidence,

were of short duration and are considered related to the surgical procedure including anaesthesia and/or post-surgery immobilization. Animals treated with autologous cells showed no major differences concerning macroscopic and microscopic findings of the femoral condyle as compared to control animals.

As part of the goat study, the macroscopic, histological and biochemical composition of the synovium and synovial fluid was investigated 10 and 52 weeks post-implantation with particular attention to inflammation and ectopic cartilage or bone formation. At 10 weeks, ca. 70 % of all animals showed various degrees of synovitis.

In a feasibility study in sheep the majority of the animals showed penetration of the transplanted cells in subchondral bone. In two cases complete penetration of underlying bone marrow was observed. Similar findings were observed in long-term studies in goats. In addition these animals showed complete penetration of underlying bone marrow.

The observed synovitis and the reported penetration of the transplanted cells in the subchondral bone have been identified in the RMP as potential safety concerns related to the use of the product that warrant a specific statement under section 5.3 'Preclinical safety data' of the SmPC.

None of these potential concerns are found to have an impact on the safe clinical application of ChondroCelect. Further risk minimization actions or additional non-clinical data are not considered necessary.

No effects on body systems or systemic toxicity was seen in the mice, sheep or goats as expected for this kind of autologous cell therapy medicinal product applied locally in this compartment.

- Repeat dose toxicity (with toxicokinetics)

As the observation period of the single dose studies described above were up to 12 weeks in mice, 14 weeks in sheep and 53 weeks in goats, these studies are considered to be sufficient to assess the long-term effects of ChondroCelect. Therefore the omission of *repeat-dose toxicity* studies is in line with the EMEA guideline EMEA/CHMP/410869/2006.

- Genotoxicity

The omission of *genotoxicity studies* in the development program for ChondroCelect is in line with EMEA/CHMP/410869/2006.

- Carcinogenicity

In order to address the carcinogenic potential of ChondroCelect, the Applicant performed an *in vitro* study to evaluate senescence of human articular chondrocytes after serial passaging, using ChondroCelect culture conditions. Cells were kept beyond the routine cell culturing as suggested in EMEA/CHMP/410869/2006.

The results provide sufficient evidence that immortalisation of human chondrocytes during limited time in *in vitro* culture conditions would not occur, and that the risk of tumorigenic growth is negligible.

In view of these results, the absence of standard carcinogenicity studies was considered to be acceptable.

- Reproduction Toxicity

Taking into account the nature of the product and its intended clinical use the risk for reproductive and developmental toxicity is considered to be negligible.

Therefore, the omission of *reproductive and developmental toxicity* studies in the development program for ChondroCelect is acceptable and in line with the EMEA guideline EMEA/CHMP/410869/2006.

- Local tolerance

Local tolerance was an integral part of the toxicological studies. Therefore, no dedicated *local tolerance* studies with ChondroCelect. were deemed necessary. Pharmaco-toxicological studies in the

orthotropic animal model showed that implantation of human or allogeneic chondrocytes causes an immune response to the CBMP, resulting in poor repair.

- Other toxicity studies

Ecotoxicity/environmental risk assessment

ChondroCelect is composed of non-modified human autologous cells. The cells administered to the patient are likely to remain in the implantation site and are not released to the environment. Therefore the use of ChondroCelect is unlikely to result in any risk to the environment, due to its nature and also because the product is not released into the environment.

Consequently, the absence of environmental studies is in line with the EMEA guideline EMEA/CHMP/SWP/4447/00.

2.4 Clinical aspects

Introduction

GCP

The GCP inspection highlighted the amount of missing data on the structural endpoint and the change to the ICRSII read-out in the pivotal study as major concerns.
Most of the concerns related to the ICRSII were resolved during the procedure. The generation of new slides from the original repair biopsy has increased the ICRSII data base from 73% to 93%. It was, however, acknowledged that the *a priori* determined primary efficacy end point, the MODs score (on the basis of which the for example *a priori* sample size calculations and power analysis were performed), was *post hoc* disregarded as invalid and a new primary end point, the ICRSII was developed within the course of the study, the conclusion being that this GCP non compliance cannot, as such, be *post hoc* rectified.

Pharmacokinetics

Studies on absorption, distribution, metabolism and excretion have not been performed Conventional ADME studies are usually not relevant for a cell based medicinal product. The body distribution/migration studies are part of the non-clinical development program. This is acceptable considering the nature and origin (autologous) of the product.

Pharmacodynamics

Conventional pharmacodynamic studies for ChondroCelect have not been performed. The pharmacodynamic parameter "histological evaluation" was part of the efficacy assessment in the phase III trial. The ChondroCelect score is a functional test which suggests a correlation between the gene expression profile of chondrocytes and hyaline cartilage formation in vivo in animal models and was used also in the phase III study (see Overview on quality and non-clinical development as regards the discussion on validity of this score). In the pivotal study a periosteal flap was used to seal the defect and maintain the chondrocyte suspension in situ.

Clinical efficacy

- Dose response study(ies)

No dose-response studies have been performed. The dose selection was based on a combination of animal studies conducted by TiGenix, published literature and experience in humans with ACI. On the basis of this information the dose of between 0.8 and 1×10^6 cells/cm^2 was used.

- Main study(ies)

Study TIG/ACT/01/2000 is a phase III, multicentre, randomized, controlled trial to compare ChondroCelect to the procedure of microfracture in the repair of symptomatic single cartilaginous lesions of the femoral condyles of the knee. This study TIG/ACT/01/2000 and its ongoing 4-year extension phase (TIG/ACT/O1/2000EXT) (both referred to as TIG/ACT/OI&EXT') were initially separate studies which were merged late in the development.

Methods

Study Participants

Patients aged between 18 and 50 years, who had a single symptomatic cartilage lesion between 1 and 5 cm^2 of the femoral condyles met the inclusion criteria.
Patients with patellofemoral cartilage lesion, osteochondritis dissecans (OCD), depth of lesion >0.5 cm, prior meniscal transplant, prior mosaicplasty and prior microfracture within last 12 month were excluded.
Patients had to agree to actively participate in a strict rehabilitation protocol and follow-up program.

Treatments

Microfracture is considered an effective standard treatment for smaller femoral cartilage lesions according to currently available literature data, and is an acceptable control therapy.

Objectives / Outcomes / Endpoints

The primary objectives of this study were changed in August 2006, after end of the initial study period. The primary objective of the original protocol was to show superiority in structural repair at 12 months compared to the control group. However, CHMP Scientific Advice suggested that a clinically meaningful primary endpoint should be used. Therefore, the Applicant decided to follow CHMP Scientific Advice and to select overall KOOS (The Knee Injury and Osteoarthritis Outcome Score, 1998) for the second primary efficacy endpoint. This questionnaire-based endpoint has five separately scored subscales: 1) pain; 2) other symptoms such as swelling, restricted range of motion and mechanical restrictions; 3) function in daily living; 4) function in sport and recreation; 5) knee-related Quality of Life. At the ad-hoc expert group that was convened on 13th of October 2008, the experts confirmed that patient-reported outcomes should be the primary outcome measure in studies in orthopaedics and sports medicine, and that the KOOS is one of the most meaningful clinical endpoints to date.

The modified **primary objectives** of the study included the following structural and clinical objectives: To show an advantage of ChondroCelect compared with microfracture in the treatment of symptomatic cartilaginous defects of the femoral condyle of the knee by demonstrating superiority on the structural repair (histology) endpoint at 12 months and non-inferiority on the clinical endpoint (change from baseline in KOOS) for the average of the 12- to 18-months follow-up data. Due, firstly, to the more complex nature of ACI compared with microfracture and associated safety issues, secondly, due to the fact that very limited data exists on the efficacy of MF, in particular in the long term setting, and thirdly, due to the fact that the relevance, both short term and long term, of the structural findings has not been established, CHMP Scientific advice (EMEA/151996/2006) recommended that it is of importance to establish the superiority of ACI.

Secondary objectives were to assess the difference between ChondroCelect and microfracture at 12 months in terms of the following structural outcome parameters: ICRS II sub-scales, MRI measurements and ICRS Visual Histological Assessment Score.
Primary efficacy parameters were

- The sum of histomorphometric scores on safranin-O and collagen II staining (sum of two ratios) and the mean Overall Histology Assessment score at 12 months.

- The change from baseline in Overall KOOS score averaged over 12 and 18 months.

Sample size and Randomisation

Sample size calculation was based on the score component of the MODS (primary endpoint according protocol). Due to a lack on information on the expected variability, the sample size was determined using only the categorization (success/failure) element of the MODS. Anticipating a 30% success rate for the microfracture group and a 60% success rate for the ChondroCelect procedure, it was calculated that with 112 (56 per group) a one-sided test at the 2.5% level would have 90% power to detect such a difference. However, this primary endpoint was disregarded as invalid and a new histological end point was developed during the conduct of the study.

Randomisation was performed via a central IVRS.

Blinding (masking)

All clinical assessments were performed by independent evaluators at each site. Two central histopathologists who were blinded to the treatment allocation completed the histopathological and histomorphometric assessments of the biopsies. The scoring of the MRI scans was also performed centrally by two independent musculoskeletal radiologists, who were blinded to the treatment allocation. The randomisation was performed using the "minimisation" method, in order to balance groups for the most important prognostic factors.

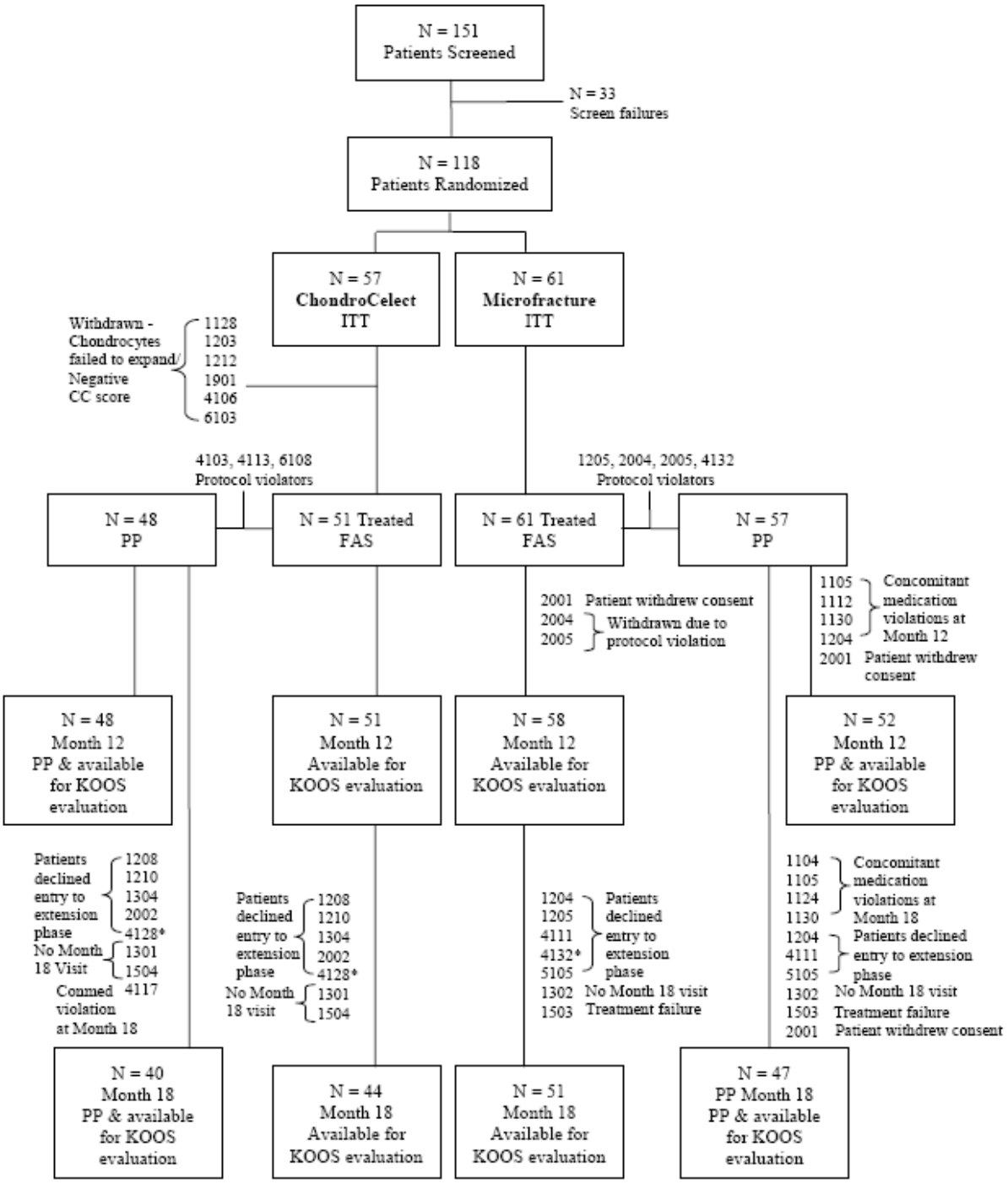
Statistical methods

All analyses were performed for the FAS population (i.e. all patients randomized who underwent the surgical procedure). For the primary efficacy parameter additional (secondary) analyses were to be performed for the ITT and PP population respectively.

RESULTS

Participant flow

Figure 6 Patient Population Flowchart



* Patients declined entry to extension phase due to re-intervention

Recruitment

The study was performed in 13 centres in 4 countries.

Conduct of the study

The initial 12-months trial protocol was dated 22 October 2001. Seven protocol amendments were subsequently implemented. The first two amendments were implemented prior to inclusion of any patient.

Baseline data

The randomisation to ChondroCelect and microfracturing groups was successful for age (mean age 33.9 years and 33.9 years, respectively, gender (61% and 67% males), and weight (mean 78.1 kg and 80.6 kg). There was a higher proportion of patients with a BMI >30 kg/m2 in the microfracture group than in the ChondroCelect group (9.8% versus 5.3%) and a slightly higher proportion of patients in the microfracture group whose onset of symptoms was acute compared to the ChondroCelect group. The median duration of time since onset of knee injury was slightly longer in the ChondroCelect group than in the microfracture group (2.0 years versus 1.6 years). The presence of concomitant cartilage lesions was comparable in both groups (30% versus 25%). More patients in the ChondroCelect treatment group, compared to patients in the microfracture group, had undergone previous knee surgery (88% versus 77%).
The lesions of the femoral condyle that were treated with ChondroCelect or microfracture were ICRS grade III or IV, except for one patient with a grade II lesion in the ChondroCelect group. Thirty per cent (17/57) of patients in the ChondroCelect group and 25% (15/61) of patients in the microfracture group had additional concomitant cartilage lesions (data from Clinical Overview). The mean surface area of cartilage defect post-debridement was similar in both treatment groups (mean 2.64 and 2. 44, respectively).

Numbers analysed

The patient disposition is seen in the Table below.

Table 8 Patient Populations

Population	Number of Patients (%)		
	ChondroCelect (N=57)	**Microfracture (N=61)**	**Total (N=118)**
Intent-to-Treat	57 (100%)	61 (100%)	118 (100%)
Safety	57 (100%)	61 (100%)	118 (100%)
Full Analysis Set	51 (89%)	61 (100%)	112 (95%)
Per Protocol	48 (84%)	57 (93%)	105 (89%)
Available for KOOS & without any protocol violations at Month 12	48 (84%)	52 (85%)	100 (85%)
Available for KOOS & without any protocol violations at Month 18	40 (70%)	47 (77%)	87 (74%)

Source data: Table 1.1.1 and Listing 1.1.

The full analysis set (FAS) was used for the main efficacy analysis. Six patients in the ChondroCelect group were excluded from FAS because acceptable products could not be prepared from the biopsies. All patients were included in the safety analysis. Seven patients in both groups were lost for follow up between months 12 and 18.

Outcomes and estimation

Primary endpoints

The results of the analysis of structural repair and of the clinical endpoint are presented in the Table below:

Parameter	Treatment	N	Adjusted Mean (SE)	Difference (95% CI)	p-value
Histomorphometric endpoint	ChondroCelect	47	1.01 (0.08)	0.26 (0.09, 0.44)	0.003
	Microfracture	54	0.75 (0.08)		
ICRS II at 12	ChondroCelect	49	55.11 (3.98)	10.92 (2.63, 19.21)	0.0103

months	Microfracture	55	44.20 (3.74))		
Change in KOOS at 12 and 18 months	ChondroCelect	51	16.18 (2.42)	1.81 (-3.28, 6.90)	-
	Microfracture	58	14.37 (2.35)		

All estimates from ANCOVA models; adjusted for age, associated lesion & location of lesion (histomorphometric & histological endpoints); adjusted for baseline Overall KOOS score, age, associated lesion(s) & location of lesion (KOOS)

In line with the testing strategy as provided with the statistical analysis plan, superiority of ChondroCelect compared to microfracture could be shown for both endpoints describing structural repair, the histomorphometric and the histological endpoint.

The average change from baseline (at month 12 and 18) total KOOS scores for the FAS population are presented in the Table below:

	ChondroCelect (N=51)	**Microfracture (N=61)**
N	51	58
Adjusted mean change from baseline [b, c] in Overall KOOS (SE)	16.18 (2.42)	14.37 (2.35)
Difference [a] (95% CI)	1.81 (-3.28, 6.90)[b]	-

Mean change in Overall KOOS from baseline to the average of 12 and 18 months

The mean change in Overall KOOS from baseline to the average of 12 to 18 months was slightly higher for patients in the ChondroCelect group than for patients in the microfracture group. The results fulfil the predefined criteria for non-inferiority in this co-primary clinical endpoint and both changes are clinically relevant (≥10 points on a scale of 0-100). No significant differences between the groups were detected in the KOOS subdomains pain and activities in daily living. No significant difference in the improvement of pain as measured by VAS was seen between the groups.

Secondary endpoints

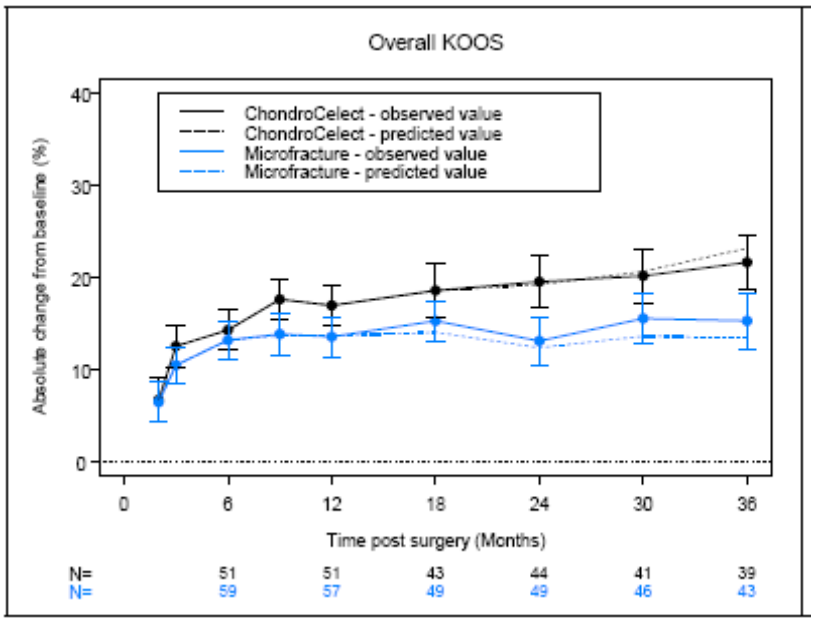
A "responder" analysis (20% improvement) showed comparable results in the groups for total KOOS and for its subdomains.

The clinical data have also been analysed when all patients reached at least 36 months follow-up. Two main analyses have been documented: analysis according to mixed linear models and further analysis of the non-inferiority over time.

During the procedure the Applicant completed and submitted statistical analysis of up to 60 month follow-up data including graphical illustrations of a mixed model with time as a continuous and as a categorical variable. However, the mean structure of the KOOS (overall as well as for each of the subdomains) is not linear over time. As a result, models anticipating a linear mean structure overestimate the effect in the CC group beyond month 24. Therefore, the time wise comparisons of treatment effects were based on a mixed model with time as a categorical variable.

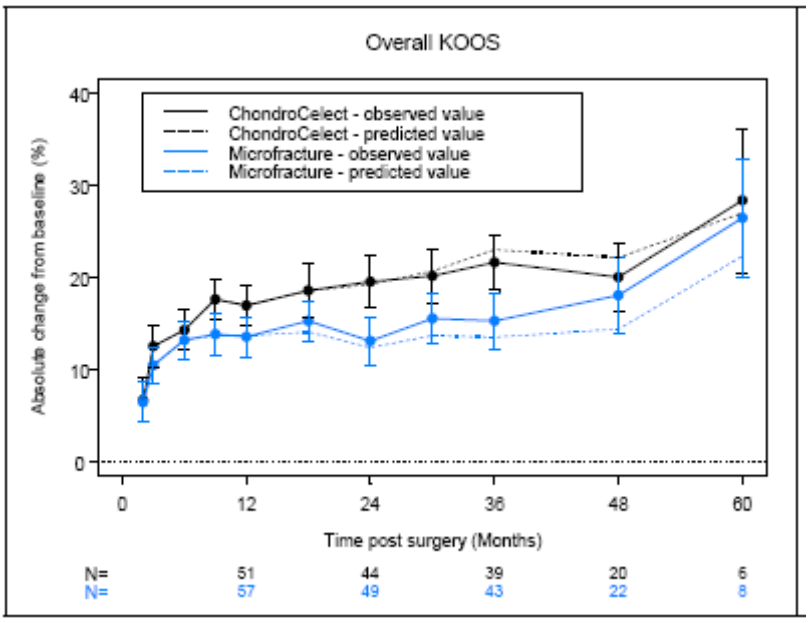
The results of the different analyses are shown below. The results from 36 months on could only be considered as descriptive, because of the small number of patients already reaching later follow-up time points.

Mixed model with time as a categorical variable – all data up to month 36



p-value for the time by treatment interaction: 0.070
Treatment effect at month 36 (CC – MI): 7.655, p-value: 0.0481

Mixed model with time as a categorical variable – all data up to month 60



p-value for the time by treatment interaction: 0.1192
Treatment effect at month 36 (CC – MI): 7.148 p-value: 0.0514
Treatment effect at month 48 (CC – MI): 6.893 p-value: 0.1493
Treatment effect at month 60 (CC – MI): 5.771 p-value: 0.4616

The additional analyses indicate that **C**hange (increase) from baseline in KOOS in the ChondroCelect group (CC) is numerically more pronounced when compared to the Microfracture group (MI). This observation is true not only for the overall KOOS but also for the subdomains. The additional mixed model analysis with time as a categorical variable does not give statistically significant differences at months 36, 48 and 60 (when considering multiplicity).
The second analysis which was performed was the further analysis of the non-inferiority over time. The Applicant has calculated the 95% CI for the treatment difference for each consecutive visit (i.e.

months 2, 3, 6, 9, 12, 18, 24, 30, 36, 48, and 60) in order to assess a possible non-inferiority of CC (compared to MF). For data missing as a result of treatment failure, a last observation carried forward (LOCF) approach was applied.

The results for the change from baseline over time in Overall KOOS are presented in the table below.

Table 1 Overall KOOS – change from baseline till 36 months

Time point	Treatment group	N	Mean	Difference	LS mean	SE	Difference	95% CI
2 months	CC	51	6.66	0.24	4.71	2.43	-2.10	-7.19, 2.98
	MF	59	6.42		6.81	2.35		
3 months	CC	51	12.51	2.07	11.65	2.30	-0.02	-4.84, 4.81
	MF	59	10.44		11.66	2.23		
6 months	CC	51	14.27	1.09	13.29	2.40	-0.89	-5.91, 4.14
	MF	59	13.18		14.18	2.32		
9 months	CC	51	17.63	3.80	15.74	2.51	1.96	-3.33, 7.25
	MF	56	13.83		13.78	2.48		
12 months	CC	51	16.96	3.42	14.64	2.55	1.55	-3.83, 6.93
	MF	57	13.54		13.09	2.48		
18 months	CC	44	18.45	2.95	19.19	2.79	0.72	-5.33, 6.78
	MF	51	15.50		18.47	2.87		
24 months	CC	45	19.38	6.29	19.65	3.28	4.46	-2.63, 11.55
	MF	52	13.09		15.19	3.29		
30 months	CC	43	20.71	5.55	18.49	3.47	3.27	-3.92, 10.46
	MF	51	15.16		15.22	3.39		
36 months	CC	41	22.14	7.45	21.25	3.60	5.42	-2.09, 12.94
	MF	50	14.69		15.83	3.48		

Source data: TIGACT01&EXT, efficacy tables – analysis of month 36 data, 11 May 2009

The data show that the (unadjusted) mean change from baseline is higher for patients in the ChondroCelect group than for patients in the microfracture group at all time points and with a gradual increase over time (which is also found in the adjusted means).
Non-inferiority of ChondroCelect compared to microfracture is confirmed at all time points as the lower limit of the 95% CI for the difference between the adjusted means is above the pre-defined delta of -9% points for all time points.
Neither BMI nor Gender have a significant influence on the overall KOOS.

Treatment failures

Treatment failure is defined as "the decision by the participating orthopaedic surgeon to proceed with a re-intervention (i.e. new procedure) on the same defect (index lesion) based on persistence or recurrence of symptoms as reported by the patient". In the context of this definition, any operation on the involved knee that involves the index lesion to a clinically relevant extent (i.e. 20% or more of its surface), or is intended as a result of clinical treatment failure is considered a re-intervention. Generally, the surgeon relies on MRI and/or arthroscopic assessment to confirm the patient's complaints are caused by failure of the therapeutic intervention on the index lesions and to exclude possible other causes (e.g. a new lesion).

After 36 months post-surgery follow-up, the total number of treatment failures is 2 for the ChondroCelect group and 7 for the microfracture group (p=0.178). However, as the inefficacy of the therapeutic procedure is considered a serious AE, since requiring a surgical re-intervention with hospitalisation, it was felt important to include all known treatment failures up to the 36 month time point and not only those that effectively occurred within a 36-months post-surgery timeframe. As a result, the cumulative number of treatment failures becomes 5 for the ChondroCelect group (9.8%) and 9 for the microfracture group (15%) (p=0.569), as outlined in the table below. These failure rates are concurring with failure rates reported in published literature on ACI and microfracture (Peterson *et al*., 2000; Peterson *et al*., 2002; Peterson *et al*., 2003; Micheli *et al*., 2006; Wood *et al*., 2006; Minas *et al*., 2009; Mithoefer *et al*., 2009).

Table 2 TIG/ACT/01&EXT – treatment failures

AEs reported over a 36 month post-operative period	**ChondroCelect**		**Microfracture**		**P-value***
	N	**%**	**N**	**%**	
Total number of treated patients	51	100%	61	100%	-
Treatment failures after 36 months follow-up	2	3.9%	7	11%	0.178
Treatment failures - all cases known at time of 36 months database cut-off	5	9.8%	9	15%	0.569

As is shown in table 2, fewer re-interventions for inefficacy were reported in patients treated with ChondroCelect compared to microfracture (i.e. 5 out of 51 patients [9.8%] versus 9 out of 61 patients [15%], respectively). Treatment failures in the ChondroCelect treated patients were all associated with some degree of periost loosening or graft delamination whereas in the microfracture group the majority of re-interventions were reported to be associated with insufficient or inadequate repair tissue formation. As the use of a biological membrane is expected to result in less friction at the graft surface (i.e. less hypertrophy and less crepitations), the use of a biological membrane instead of periost may possibly reduce the frequency of treatment failures upon treatment with ChondroCelect.

Ancillary analyses

- Analysis performed across trials (pooled analyses and meta-analysis)

N/A

- Clinical studies in special populations

N/A

- Supportive study(ies)

Prospective, long-term follow-up study of patients in the Belgian Armed Forces treated with ChondroCelect (TIG/ACT/02)

Methodology and baseline data

This study is a prospective, non-comparative, open-label study of 2 to 5 years' duration in 20 patients with single and multiple symptomatic cartilage defects, in any location of the knee, who underwent CCI using ChondroCelect. Patients satisfying the inclusion and exclusion criteria were administered ChondroCelect during an arthrotomy which occurred approximately 4 weeks after the arthroscopic procurement of cartilage. Approximately 1 week following arthrotomy, they were discharged from the hospital and invited for regular follow-up visits for up to 5 years after CCI. Clinical outcome, knee pain and activity levels are assessed before (pre-operatively) and after CCI by the KOOS, visual analogue scale (VAS) for pain, Activity Rating Scale (ARS) and military tests for physical fitness (MTLG). Data on pre-defect activity levels were available for the ARS and were expected to be available for MTLG.
Secondary objectives of this study included to assess the extent to which the ARS and MTLG scores return to their pre-defect levels within a 5-year post-operative follow-up period. An additional efficacy endpoint listed in the SAP is to assess the change from preoperative baseline in MRI measurements. A final objective is to assess the safety of CCI with ChondroCelect in this specific patient population with single or multiple symptomatic cartilage defects in the knee of any location.
Patients with symptomatic cartilage defects in the knee of any location were eligible for inclusion if they, were between 18-50 years of age, had a total cumulative cartilage defect between 1 and 21 cm2 and agreed to adhere to the rehabilitation regimen and the restrictions with regard to concomitant medication.
The study population enrolled was characterized by a male predominance (80%), a relatively high age (65% ≥40 years), relatively high body mass index (BMI) (60% >25 kg/m2), and a relatively recent onset of symptoms (mean: 0.9 years; median 0.5 years, range: 0 - 4 years). A femoral cartilage lesion was reported in 95% (19/20) of patients, a patellar lesion in 40% (8/20) and a tibial lesion in 15% (3/20). A total of 35 lesions were reported in 20 patients. The majority of patients had only one lesion (60%;12/20), whilst the remaining patients had two (10%; 2/20), three (25%; 5/20) or four (5%; 1/20) lesions. One of the patients with three lesions and the patient with four lesions each had three lesions treated with CCI. All other patients (18/20; 90%) had only single lesions treated with CCI. Of the 7 patients with multiple lesions who did not have all their lesions treated with CCI, four had their other lesions treated with either shaving (4 lesions in 3 patients) or microfracture (1 lesion in 1 patient) and three had untreated lesions.
Of all reported lesions, 80% were reported to be of ICRS Grade III or IV. Of 24 femoral lesions reported in 19 patients, 21 were treated with CCI. All femoral lesions were ICRS grade III-IV. Two of the femoral lesions not treated with CCI were ICRS Grade IV and were located on the trochlea, one of these had been treated with microfracture at arthroscopy; the third untreated femoral lesion was also located on the trochlea and was shaved at arthroscopy (lesion grade unknown). Of 8 patellar lesions reported in 8 patients, three were treated with CCI (one ICRS grade II, one ICRS grade III and one ICRS grade IV). Of the five untreated patella lesions, three were ICRS Grade I and two were ICRS Grade III. One of the grade I patella lesions not treated with CCI and one of the grade III patella lesions not treated with CCI had been shaved at arthroscopy. Three tibial lesions were reported in 3 patients, none of which were treated with CCI. Two of the tibial lesions were ICRS grade II and one was ICRS grade II to III. One of the grade II tibial lesions had been shaved at arthroscopy. Of all enrolled patients, 60% (12/20) had undergone previous cartilage repair surgery at least once (45% [9/20] debridement, 10% [2/20]) microfracture, 5% [1/20]) abrasion arthroplasty, and 5% [1/20]) multiple osteochondral autologous grafts), 60% had a previous meniscus operation, and 20% had previous ligament surgery at baseline. Two patients (9%) had an ACL repair and five patients (23%) had meniscus surgery during the arthroscopy performed for the harvest biopsy. The lesion size treated with ChondroCelect was 2.33 cm² (SD 1.16; range, 0.8 - 9.2 cm2).

Results

Table 9-1 Patient Disposition for FAS Population for the First 24 Months Following CCI with ChondroCelect

	Baseline	At Month 3	At Month 6	At Month 12	At Month 18	At Month 24
Number of patients on-going in study	20	20	19[a]	17	13[b]	9
Number of patients yet to reach timepoint	0	0	1	2	6	10
Number of patients withdrawn since preceding visit	-	0	0	1	0	0

Data source: Table 1.3 and Listings 1.1, 1.3 and 4.1.1 Appendix C.

At 24 months following CCI, the patients' clinical status was improved compared to baseline: mean change in Overall KOOS 28.3 [95% CI 11.28, 45.1; n=9]); VAS (mean change -37.3 [95% CI -63.2, -11.5; n=9]); ARS total score at 24 months was 1.0 (95% CI: -0.2 to 2.2; n=7) indicating a trend towards improved.

	Mean Change in Overall KOOS from Pre-Operative Baseline				
	At Month 3	At Month 6	At Month 12	At Month 18	At Month 24
N	17	17	16	13	9
Mean (95% CI)	0.5 (-9.5, 10.4)	15.1 (4.5, 25.7)	18.3 (7.7, 28.9)	23.5 (10.3, 36.7)	28.2 (11.3, 45.1)

The percentage of patients with asymptomatic knees (patient categorization derived from KOOS scores) was increased from none at baseline (0/19 patients) to 38% at Month 18 (5/13 patients) and 56% at Month 24 (5/9 patients).

	Mean Change in VAS for Pain Severity from Pre-Operative Baseline				
	At Month 3	At Month 6	At Month 12	At Month 18	At Month 24
N	19	18	17	13	9
Mean (95% CI)	-6.6 (-26.7, 13.5)	-24.3 (-43.8, -4.9)	-28.3 (-45.2, -11.1)	-39.5 (-58.0, -20.9)	-37.3 (-63.2, -11.5)

There was a trend towards an improvement in the patients' activity level at 18 and 24 months compared to pre-operative baseline, although their activity level remained below their pre-defect levels. The data on MTLG were insufficient to draw any conclusion.

		Mean Change in ARS Total Score			
Baseline		At Month 6	At Month 12	At Month 18	At Month 24
Pre-Defect	N	11	14	11	8
	Mean (95% CI)	-4.5 (-8.6, -0.3)	-5.4 (-8.0, -2.7)	-3.9 (-6.8, -1.1)	-4.0 (-7.8, -0.2)
Pre-Operative	N	11	14	11	7
	Mean (95% CI)	0.0 (0.0, 0.0)	0.1 (-0.5, 0.7)	0.8 (-0.3, 2.0)	1.0 (-0.2, 2.2)

Overall discussion on Efficacy

The efficacy evaluation of ChondroCelect is based on one pivotal study. At 12 months post-surgery, structural assessments (histology and MRI) were performed, and at 12 to 18 months post-surgery the clinical outcome was assessed. In line with the testing strategy as provided with the statistical analysis

plan, superiority of ChondroCelect compared to microfracture could be shown for both endpoints describing structural repair
The ICRSII had been developed within the trial, and the validity of this new tool had not been assessed prior to starting the trial. This finding could not be corrected post-hoc. However, the need to develop a suitable assessment method for structural repair was acknowledged, as well as the fact that the new ICRSII score was developed in a blinded manner.
A further issue was that many of the tissue sections could not be assessed which lead to 20% of missing data. In the course of the procedure the missing data were provided, which strengthened the superiority claim of structural repair.

With respect to the clinical component (change in overall KOOS) non-inferiority was proven. The clinical non-inferiority in the KOOS at 12-18 months was explained by the fact that cartilage requires a longer time to be repaired, given the bradytrophic nature of human joint cartilage and the long time required for differentiation and functional repair. However, statistically significant superiority over microfracture at later time points could not be shown, although the formal requirements to demonstrate non-inferiority at 36 months are fulfilled.

In the supportive study TIG/ACT/02 the results of the informal interim analysis show a trend towards clinical benefit. However, only 9/20 patients reached the 24 month time point by the time of the analysis and were assessed for efficacy. The contribution of this study to the benefit/risk analysis of the product is small.

Clinical safety

- Patient exposure

A total of 463 patients have been exposed to ChondroCelect. In the two clinical studies 71 patients w were treated with ChondroCelect, and 61 underwent microfracture treatment. Twenty-two (22) patients were included in the expanded access program and 370 patients were included in the compassionate use program. Safety data from 334 patients are available from the compassionate use program. In both the clinical studies and programs, the absolute dose of ChondroCelect received was determined by the size of the lesion(s) treated.

- Adverse events

First, the overall frequencies of adverse events (AEs) between the two groups are summarised. Then, those AEs that occurred more frequently in the ChondroCelect group as compared to the microfracture group are discussed.

Comparative AE frequencies (TIG/ACT/01&EXT – ChondroCelect vs microfracture)

Table 3 provides an overview of the frequencies of treatment-emergent adverse events (TEAEs) in both treatment groups in the pivotal clinical trial (ChondroCelect and microfracture). Overall, patients treated with either ChondroCelect or microfracture show a similar frequency pattern of TEAEs. A slightly larger proportion of the patients in the ChondroCelect group experienced at least one TEAE when compared to microfracture (98% versus 82%); a similar pattern is also observed when only the related TEAEs are considered (78% versus 62%). The number of patients that experienced a severe TEAE, however, is very similar in both treatment groups. In contrast, the number of serious adverse events (SAEs) or the number of patients with an adverse event (AE) leading to discontinuation, was higher in the microfracture group (respectively 9.8% versus 18%, and 0% versus 4.9%). The totality of these data suggests that, despite the observed excess of TEAEs in the ChondroCelect group, the patient's functionality was not mayoral impacted. There is thus no indication that patients treated with ChondroCelect in the 2-step ACI procedure are significantly more impaired by AEs than patients treated by a 1-step microfracture technique.

Table 3 TIG/ACT/01&EXT - Overall comparative AE frequencies

AEs reported over a 36 month post-operative period°	ChondroCelect		Microfracture	
	N	%	N	%
Total number of treated patients	51	100%	61	100%
Patients with at least one TEAE	50	98%	50	82%
Patients with at least one severe TEAE	14	27%	15	25%
Patients with at least one related TEAE	40	78%	38	62%
Patients with at least one treatment-emergent SAE	5	9.8%	11	18%
Patients with at least one AE leading to discontinuation	0	0.0%	3	4.9%

Reference: Database cut-off TIG/ACT/01&EXT (13-Feb-2008)
° AEs are presented as number of patients experiencing at least one AE

A similar pattern in frequencies of TEAEs between both treatment groups is further confirmed when the AEs are summarised by body system (Table 4). Up to the 36 months time point, the highest incidence of TEAEs in both treatment groups were observed in the following four body systems: i) Musculoskeletal & Connective Tissue Disorders, ii) Infections and Infestations, iii) Injury, Poisoning & Procedural Complications, and iv) General Disorders & Administration Site Disorders. The incidence in the Musculoskeletal & Connective Disorders as well as in the Injury, Poisoning & Procedural Complications body system was higher in the ChondroCelect group as compared to the microfracture group (respectively 92% versus 77%, p=0.038; and 41% versus 25%, p=0.070). These observed differences relate to some specific AEs in the ChondroCelect group, and will be further discussed here below. For all other body systems, the frequency in AEs was quite similar between both treatment groups and no statistical differences could be found.

Table 4 TIG/ACT/01&EXT – Summary of treatment-emergent AEs by body system

AEs reported over a 36 month post-operative period°	ChondroCelect		Microfracture		P-value*
	N	%	N	%	
Total number of treated patients	51	100%	61	100%	-
Musculoskeletal & Connective Tissue Disorders	47	92%	47	77%	0.038
Infections & Infestations	30	59%	33	54%	0.703
Injury, Poisoning & Procedural Complications	21	41%	15	25%	0.070
General Disorders & Administration Site Disorders	18	35%	15	25%	0.298
Gastrointestinal Disorders	13	25%	11	18%	0.364
Nervous System Disorders	9	18%	18	30%	0.185
Psychiatric Disorders	9	18%	9	15%	0.798
Skin & Subcutaneous Tissue Disorders	6	12%	4	6.6%	0.508

Surgical & Medical Procedures	5	9.8%	3	4.9%	0.465
Investigations	4	7.8%	6	9.8%	0.753
Vascular Disorders	4	7.8%	5	8.2%	1.000
Cardiac Disorders	3	5.9%	1	1.6%	0.329
Immune System Disorders	2	3.9%	3	4.9%	1.000
Respiratory, Thoracic & Mediastinal Disorders	1	2.0%	5	8.2%	0.217

Reference: Database cut-off TIG/ACT/01&EXT (13-Feb-2008)
° AEs are presented as number of patients; an AE is counted only once per patient
* Comparison of treatment groups by Fisher's exact test

When applying a conservative statistical significance of $p<0.1$, a selection of those AEs that are more frequently observed in the ChondroCelect group as compared to the microfracture group up to the 36 months time point is obtained. These are summarised in Table 5. As could be expected, four of these selected AEs categorised in the Musculoskeletal & Connective Tissue Disorders group (i.e. cartilage hypertrophy, joint swelling, joint crepitation, and joint effusion), whereas influenza-like illness is categorised in the General Disorders & Administration Site Disorders group (of note: this body system also includes the treatment failures, which will be discussed in a later section). Graft complication is categorised in the Injury, Poisoning & Procedural Complications group.

The vast majority of the ChondroCelect specific AEs all occurred during the first 18 months post-surgery, with the exception of joint effusion (Table 5). Each of these events will be discussed in further detail in the paragraphs below.

Table 5 TIG/ACT/01&EXT – ChondroCelect specific AEs

AEs reported post-operatively°	**Period between 0-18 months**		**Period between 0-36 months**		
	CC (N=51)	**MF (N=61)**	**CC (N=51)**	**MF (N=61)**	**P-value***
Cartilage hypertrophy	14	8	14 (27%)	8 (13%)	0.093
Joint swelling	11	3	11 (22%)	4 (6.6%)	0.026
Joint crepitation	7	3	9 (18%)	4 (6.6%)	0.082
Joint effusion	4	5	12 (24%)	6 (9.8%)	0.070
Influenza-like illness	4	0	4 (7.8%)	0 (0.0%)	0.040
Graft Complication	3	0	3 (5.9%)	0 (0.0%)	0.091

Reference: Database cut-off TIG/ACT/01&EXT (13-Feb-2008)
° Selected based on $p<0.1$, AEs are presented as number of patients; an AE is counted only once per patient
* Comparison of treatment groups by Fisher's exact test

Symptomatic cartilage hypertrophy

Symptomatic cartilage hypertrophy is an undesirable AE that may result in physical impairment requiring surgical arthroscopic intervention. Symptomatic cartilage hypertrophy is generally resolved after arthroscopic shaving during day-care arthroscopy.
The events of cartilage hypertrophy included both those events that were symptomatic and those that were asymptomatic. The reporting of the latter type of (asymptomatic) hypertrophy occurred mostly at 1 year, as it was observed at the 12-month arthroscopic endpoint biopsy procedure related to the clinical protocol.

Of the 14 ChondroCelect-treated patients who had AEs of cartilage hypertrophy recorded, 7 had symptomatic AEs (7/51 [14%]). The other 7 patients had AEs that were asymptomatic. In the microfracture group, 7 of the 8 patients had asymptomatic AEs of cartilage hypertrophy and one was symptomatic (1/61 [2%]). The difference between the two treatment groups of the clinically relevant symptomatic hypertrophy is statistically significant ($p = 0.022$). It is worth noting that the majority of these events occurred in the first 18 months post-surgery, indicating that this event is related to the regenerative phase of the repair tissue. All reported AEs of cartilage hypertrophy were mild or moderate in severity in both treatment groups. None was recorded as severe and none was reported as serious.

In the pivotal clinical trial, a periosteal flap was used to cover the ChondroCelect implant as this was at that time the standard surgical procedure. However, the use of a periosteal flap to cover the cultured chondrocytes is also generally considered to involve a risk of cartilage hypertrophy. Indeed, literature data indicate that tissue hypertrophy can be related to the periosteal flap that is used to cover the defect before injection of the cells (Gooding *et al*., 2006). In recent publications, the potential risk of hypertrophy was reported to be reduced with the use of biological membranes without periosteal cells (Haddo *et al*, 2004; Gooding *et al*., 2006; Steinwachs and Kreuz, 2007). In current clinical practice, the use of a periosteal cover has decreased over the last years in favour of the use of biological membranes. The preference for collagen membranes was also confirmed by the experts in the ad-hoc scientific advisory group organised by EMEA on October 13, 2008. It is anticipated that the frequency of hypertrophy as observed in the clinical trial can be reduced when a biological membrane is used., This is supported by a comparison of the symptomatic hypertrophy frequency observed in the pivotal trial population (7/51 patients, i.e. 14%) and the patients treated under compassionate use (6/334 patients, i.e. 1.8%).

Joint swelling
The reported frequency of joint swelling is higher after ChondroCelect than after microfracture and is mainly explained by the arthrotomy performed for the ChondroCelect implantation. Knee swelling suggests the accumulation of fluid in and/or around the knee. It is a well-described symptom after arthrotomy as a result of the inflammatory synovial reaction due to incision (Muckle, 1984). This is further confirmed by analysing the temporal relationship of the reported joint swelling events with surgery, showing a high frequency and a significant difference with microfracture in the first weeks after the intervention. 7 of the 11 patients reported with joint swelling after ChondroCelect experienced the AE in the first 4 weeks after intervention, compared to none after microfracture ($p=0.003$). This earlier onset in the ChondroCelect group is linked to the arthrotomy procedure. After this initial 4-week post-operative period, no significant differences between the 2 groups were reported (i.e. 3 patients in the ChondroCelect group versus 4 patients in the microfracture group). Post-operative swelling is not associated with a significant risk and is temporary. No events of joint swelling were recorded as severe during the study, and none was reported as serious.

Compassionate use program

Comparison of TIG/ACT/01&EXT and compassionate use AEs

In Table 6, a comparison is made between the safety results of the pivotal trial population and the patients treated under compassionate use, this latter population being considered to be more representative for the real-life situation. In this table, only those AEs that are considered related to

ChondroCelect or to the surgical intervention are reported. Overall, the frequencies of AEs are consistently lower in the compassionate use population as compared to the TIG/ACT/01&EXT population. This is likely explained by a relative underreporting of AEs in the real-life situation, outside the controlled environment of a clinical trial. Assuming a 50% underreporting rate, the overall frequency for most of the reported AEs becomes similar to the frequencies observed in the clinical trial.

Table 6 TIG/ACT/01&EXT and CUP – comparison of most frequent related† AEs

AEs reported post-operatively°	**TIG/ACT/01&EXT**		**CUP**	
	N	**%**	**N**	**%**
Total number of patients	51	100%	334*	100%
Patients with at least one related AE	40	78%	155	45%
Arthralgia (knee pain)	24	47%	67	20%
Cartilage hypertrophy - symptomatic (Total)	7 (14)	14% (27%)	6	1.8%
Joint crepitation	9	18%	17	5.1%
Joint swelling	7	14%	23	6.9%
Joint effusion	5	9.8%	24	7.2%
Treatment failure	5‡	9.8%	9	2.7%

Reference: TIG/ACT/01&EXT database cut-off 13 February 2008 (36 months), CUP database cut-off 7 January 2009

° AEs are presented as number of patients; an AE is counted only once per patient

***** Number of patients contributing to the safety population at time of database cut-off (7 January 2009)

† Related to ChondroCelect or surgical intervention

‡ All cases known at the time of the 36 months database cut-off

As can be seen from the table, knee pain (Arthralgia) is the most frequently reported treatment-related AE in both the pivotal study (24/51 patients, i.e. 47%) and the CUP (67/334 patients, i.e. 20%). In the pivotal study, a similar frequency of Arthralgia was observed in the ChondroCelect (47%) and microfracture (43%) groups up to the 36 months cut-off. This AE was not discussed in the overall analysis of the TIG/ACT/01&EXT data as the difference between the two treatment groups was not significant (p=0.704).

The incidence of symptomatic cartilage hypertrophy was reduced to 1.8% in the CUP patients compared to 14% in the pivotal study. It can be assumed that the 6 reported AEs in the CUP patients are all symptomatic AEs, as these patients did not undergo an endpoint biopsy. In the patients treated under compassionate use, ChondroCelect has been covered with a biological membrane (ChondroGide®) in the majority of the patients in contrast to the use of a periosteal flap in the pivotal trial. The lower incidence of cartilage hypertrophy observed in the CUP is in line with other reports published in the literature on low hypertrophy rates with a biological membrane (Haddo *et al*, 2004; Gooding *et al*., 2006; Steinwachs and Kreuz, 2007), and illustrates that a similar benefit of using a biological membrane can also be obtained for ChondroCelect.

The relatively high incidence of joint effusion in the compassionate use patients (7.2% of patients in the CUP as compared to 9.8% in the pivotal trial) is considered to result from the fact that in this population more salvage cases with more complex concomitant knee pathology have been treated. A detailed assessment of these cases reveals that in all patients but 2, the effusion was considered to be

of mild (14) or moderate (8) intensity. None of the 24 cases was reported to be serious. In 15 cases, the joint effusion was considered to be related to the surgical procedure (3 unknown, 6 not related to surgery), and in 7 patients the event was considered to be related to ChondroCelect.

For all other AEs, similar or slightly lower frequencies are observed when considering a 50% underreporting in the CUP (i.e. joint crepitation [18% versus 5.1%], joint swelling [14% versus 6.9%], and treatment failures [9.8% versus 2.7%]).

- Serious adverse event/deaths/other significant events

Twenty four serious adverse events (SAEs) were reported in 16 patients (8 SAEs in 7 patients in the TIG/ACT/01&EXT study and 16 SAEs in 9 patients in the TIG/ACT/02 study). Only one of the 24 cases was considered to be related (possibly) to ChondroCelect. In this case, the ChondroCelect transplant was considered to have failed possibly because of loosening of the periosteal flap, and was removed; microfracture was subsequently performed. The majority of cases (14/24; 58%) were considered to be unrelated to the study procedure. In the pivotal TIG/ACT/01&EXT there were 10 SAEs in 8 patients recorded in the microfracture group. All cases recorded for microfracture-treated patients were considered either unrelated or unlikely to be related to the surgical procedure.

From additional safety information gathered from the EAP and compassionate use program, there have been 26 SAEs reported for 18 patients (17 SAEs in 10 of the 22 patients in the EAP, 9 SAEs in 8 of the 163 patients in the compassionate use program for whom safety data are available). Of these, only one case was considered by the surgeon to be related (possibly) to ChondroCelect. In this case the patient experienced a deficit in knee mobilisation of moderate severity, approximately 2 months after the implantation of ChondroCelect (secured with ChondroGide). The patient underwent a procedure to mobilize the knee under anaesthesia.

There were no patient deaths recorded during the study. No patients are recorded as being discontinued from the study due to SAEs.

- Laboratory findings

No laboratory findings related to the ChondroCelect treatment were reported.

- Safety in special populations

Twelve paediatric patients were treated in the compassionate use program with ChondroCelect.

Five of the 11 (45%) paediatric patients had no reported AE. The remaining 6 paediatric patients reported a total of 11 AEs. None of the AEs reported was considered serious. None of the AEs was considered to be related to ChondroCelect; most were considered to be related to surgery (7/11 events; 64%). Most AEs (8/11; 73%) were of mild or moderate intensity and did not require any intervention or medical therapy. Three events were recorded as being of severe intensity, one event of muscle atrophy (n=2) and arthralgia (n=1).
There was one pregnancy during ChondroCelect therapy. The mother developed pre.eclampsia, had a premature birth of a normal child. The use of ChondroCelect is not recommended during pregnancy, mainly because of the surgical procedures.

- Safety related to drug-drug interactions and other interactions

No investigations have been performed

- Discontinuation due to adverse events

See efficacy part for discontinuation due to treatment failure.

- Post marketing experience

N/A

- Other relevant safety information

A clinically meaningful event that is not reported in the classical AE capturing relates to the impact of the procedures on the subchondral bone. Detailed assessment of the MRI data of the pivotal clinical trial revealed that treatment with ChondroCelect resulted in less subchondral bone reaction as compared to microfracture. A difference of 0.45 on the 0-3 global scale was observed in favour of ChondroCelect (p=0.0559). In addition, the incidence of subchondral bone plate elevation was shown to be higher in the microfracture group when compared to the ChondroCelect group (51.5% compared to 25%, respectively).

The difference in subchondral bone reactions relates to the difference in surgical intervention. In microfracture, chondral defects are treated by recruiting blood and cell populations including mesenchymal stem cells from the underlying bone marrow. By physically disturbing the osteochondral junction (upon puncturing the subchondral bone), an osteochondral defect is de facto created, and the biology of the cartilage defect significantly altered. In contrast, in ACI cells are re-implanted into the defect without damaging the subchondral bone. The consequences of the injury and its increased subchondral bone reaction and moving up of the bone front might lead to poorer repair tissue, decreased durability of the repair tissue, and consequently increased risk of treatment failure (Mithoefer *et al*., 2009). The moving of the bone front (ultimately leading to intra-lesional osteophytes) might also have consequences for future re-interventions. Indeed, Minas *et al*., 2009 published that prior treatment affecting the subchondral bone such as microfracture increases the failure rate of subsequent regenerative procedures. Finally, the biological stability of the osteochondral junction can be of importance in the development of osteoarthritis, instability of the subchondral bone and progressive bone damage being important factors in progression to the disease (Dieppe and Lohmander, 2005; McQueen, 2007). It is also anticipated that in genetically predisposed patients, disturbing the osteochondral junction presents an additional risk for a fast progression to osteoarthritis (Luyten *et al*., 2009).

Discussion on Safety

Microfracture is performed in one arthroscopic procedure with either local (spinal) or general anaesthesia, while ACI requires 2 interventions: an arthroscopy to inspect the defect and to obtain the biopsy specimen, and open knee surgery (arthrotomy) in general anaesthesia for chondrocyte implant four weeks later. In the case of patients included in the ChondroCelect arm of the pivotal trial a second incision was made over the medial tibia to harvest the periosteal flap which was needed to cover the chondrocyte suspension.
The most likely AEs observed when treating with ChondroCelect are arthralgia, symptomatic cartilage hypertrophy, joint crepitations, joint swelling, and joint effusion. Arthralgia is an expected and common consequence of knee surgery and occurs in both treatment arms. The data provided on the CUP program as well as data from published literature suggest that it may be possible to reduce ChondroCelect-related cartilage hypertrophy by the use of a collagen membrane. This modification will reduce the potential for morbidity associated with the harvest of the periosteal flap. Joint crepitation is a mild complication and occurs also in the normal population. Joint swelling is another complication observed at a higher rate in the ChondroCelect group. It is a consequence of the arthrotomy surgical intervention and can as such not be avoided. However, it is a mild and transient complication. The incidence of joint effusion was higher after 18 months post-intervention than in the period after the surgery, and the reason for this is not completely clear. Analysis of the individual cases indicate that the underlying knee disease status as well as potentially higher physical activity rate might be related to this occurrence. The cases were not severe or serious, and do therefore not represent a major safety signal.
Overall, the safety profile of ChondroCelect is considered acceptable. Considering that cartilage hypertrophy may be reduced by use of a physical seal the main difference is related to arthrotomy and the implantation procedure. No complications were seen in relation to the arthroscopic harvest biopsy procedure.

Considering the higher treatment failures after microfracture which require more surgical re-interventions microfracture and ChondroCelect implantation have a balanced safety profile.

The CHMP was of the opinion that the indications (section 4.1 of the SPC) should be reworded as follows:

Repair of single symptomatic cartilage defects of the femoral condyle of the knee (International Cartilage Repair Society [ICRS] grade III or IV) ~~from 2 cm² onwards~~ in adults. Concomitant asymptomatic cartilage lesions (ICRS grade I or II) might be present
Demonstration of efficacy is based on a randomised controlled trial evaluating the efficacy of ChondroCelect in patients with lesions between 1-5cm^2.

The scientific reasons for this change are described below:

CHMP considered that putting a precise lower boundary would prevent physicians from treating patients on-label who have lesions in sizes formally below 2cm2, for whom, however, ChondroCelect treatment might nevertheless be medically indicated by the individual decision of the treating physician. Likewise, CHMP recognised the greater need for the treatment of larger lesions, but was concerned about the amount of data available for larger lesions. Therefore, in line with previous similar scenarios, the CHMP has taken the approach to put an indication that is permissive, but to point out the limitations of the data and to clearly inform the prescribing physician in section 4.1. A wording like this is usually perceived as stronger as compared to a wording in section 5.1, thus being more in line with the overall principles of the CAT draft opinion. This allows the physician to take an informed decision for a particular patient's situation based on the knowledge of the availability of evidence from the pivotal study.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system as agreed at CAT

The CAT considered that the Pharmacovigilance system as described by the Applicant fulfils the legislative requirements.

Risk Management Plan as agreed at CAT

The MAA submitted a risk management plan, which included a risk minimisation plan and efficacy follow-up plan.

Table Summary of the risk management plan

Important potential risks

Safety concern	**Proposed pharmacovigilance Activities** **(routine and additional)**	**Proposed risk minimisation activities** **(routine and additional)**
Partial or complete delamination of the periost flap, synovitis, subchondral bone injuries	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction	▪ Information in section 5.3 of the SPC on the findings of synovitis and subchondral bone injuries. ▪ Pro-active training in the framework of a controlled distribution system.

Safety concern	Proposed pharmacovigilance Activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
	reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	

Important identified risks

Safety concern	Proposed pharmacovigilance Activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Symptomatic cartilage hypertrophy	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	▪ Information in section 4.8 of the SPC that the event of cartilage hypertrophy can be associated with the use of a periosteal flap instead of a biological membrane. ▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.
Knee joint swelling	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.
Knee joint crepitation	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.
Joint effusion	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction	▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.

Safety concern	Proposed pharmacovigilance Activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
	reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	
Arthrofibrosis	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon	▪ Information in section 4.4 of the SPC on risks associated with concomitant knee pathologies or use outside the target population. ▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.
Ineffectiveness (treatment failure)	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon ▪ Non-interventional Post-marketing safety and efficacy study.	▪ Information in section 4.4 of the SPC on risks associated with concomitant knee pathologies or use outside the target population. ▪ Information in section 4.8 of the SPC on the observed incidence of this adverse event. ▪ Pro-active training in the framework of a controlled distribution system.

Important missing information

Safety concern	Proposed pharmacovigilance Activities (routine and additional)	Proposed risk minimisation activities (routine and additional)
Long term durability of repair and clinical data in patients with larger lesions *(from 4 cm² onwards)*, and confirmatory clinical data in patients with smaller lesions	▪ Routine pharmacovigilance ▪ Proactive training of orthopaedic surgeons and their staff on the use of the product and the associated procedures ▪ Solicited adverse reaction reporting and interaction with the surgeon based on the medical dossier ▪ Medical information and feedback to the surgeon ▪ Continued follow-up of patients of the pivotal clinical study (TIG/ACT/01&EXT). ▪ . ▪ Post-marketing safety and	▪ Post-marketing safety and efficacy study. ▪ Further efficacy data obtained in patients in a confirmatory clinical study.

	efficacy study.	

The CAT, having considered the data submitted, was of the opinion that the risk management system should be requested according to the Article 14 (2) of the Regulation (EC) 1394/2007. There are the following particular causes for concern:

- There were deficiencies in the conduct of the pre-authorisation studies and uncertainties related to the result of the submitted single pivotal trial.
- There is unknown long-term durability of the product efficacy.
- Benefit/risk of the product is significantly influenced by the level of compliance with the defined procedures throughout the treatment with ChondroCelect, from the biopsy harvest till the correct physiotherapy.

The CAT, having considered the data submitted in the application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product:

The Marketing Authorisation Holder (MAH) shall ensure that the medicinal product will be distributed only to Healthcare Establishments that meet criteria described in the Risk Management Plan.

The Marketing Authorisation Holder (MAH) shall ensure, prior to the distribution of the product to a particular Healthcare Establishment, that all surgeons and other healthcare professionals involved in the handling and administration of ChondroCelect or its components, as well as those involved in follow-up of patients treated with ChondroCelect in the Healthcare Establishment, receive training as per the educational programme described in the Risk Management Plan.

The educational programme for healthcare professionals contains the following components:

- Training material for Surgeons
- Training material for other Healthcare Professionals
- Informed consent for the patients to be signed prior to the treatment with ChondroCelect

The training materials for Surgeons shall include the following key messages and components:

- Summary of Product Characteristics
- The biopsy harvest procedure
- The surgical checklist to be completed at the operating theatre immediately prior to the first incision confirming the right patient, the right product, the right side of the implantation, and the type of biological membrane and fibrin sealant to be used in the procedure.
- The implantation procedure by knee-joint arthrotomy
- The follow-up protocol

The training material for other Healthcare Professionals shall include the following key messages and components:

- Summary of Product Characteristics
- The need for screening of donors using patient questionnaire and laboratory tests for hepatitis C, hepatitis B, HIV, and Syphilis
- The handling of the biopsy harvest
- The handling of ChondroCelect and its preparation for the implantation
- The schedule of follow-up of patients
- The recommended physiotherapy

The CAT also considered that performing of post-authorisation studies will need to be a part of the Pharmacovigilance plan and Efficacy follow-up plan presented in the Risk Management Plan.

In the Risk Management Plan, the MAH commits to confirm and extend the pivotal clinical study data with an appropriately designed trial. The design should be subject to EMEA Scientific Advice, and agreed with the CAT.

In the Risk Management Plan, the MAH also commits to further study efficacy and safety of ChondroCelect in large lesions. The design of such a study should be subject to EMEA Scientific Advice, and agreed with the CAT.

The timetable for the conduct of the studies was agreed with the Applicant. The MAH commits to submit, within two weeks after the CHMP opinion, an update to the Risk Management Plan (RMP) to include the following points:

a. Studies, and their protocol outlines as requested by CAT and CHMP to be reflected in the Pharmacovigilance plan and Efficacy Follow-up plan.

b. Timetables of the actions in the Pharmacovigilance plan and in the Efficacy Follow-up plan:

- Submission of application for Scientific Advice to EMEA regarding all interventional studies planned in the RMP – Date of CHMP opinion + 2 month
- Start of the studies in a way recommended by the Scientific Advice – within 1 year after adoption of the requested EMEA Scientific Advice
- Annual reporting providing interim analysis of safety and efficacy from the studies.

The CHMP agreed with the above

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The Applicant has made considerable progress towards the improvement of critical quality aspects in the manufacture and control of ChondroCelect. The outstanding major concerns raised during the procedure could be resolved and are now considered adequately addressed with data and/or follow-up commitments. A number of control measures as well as adequate tools to monitor functionality of the cells and to perform a robust process validation have been successfully implemented. Some activities related to the specification limits are still under development and await their final implementation. The Applicant has committed to address these minor outstanding issues through follow-up measures.

In conclusion, information on development, manufacture and control of ChondroCelect Active Substance and finished product have been presented in a satisfactory manner. The results of the tests carried out indicate satisfactory consistency and uniformity of the manufacturing process and finished product, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

Non-clinical pharmacology and toxicology

Primary pharmacodynamic studies were performed in two animal models, an ectopic model in nu/nu mice and an orthotopic model in goats. These pharmacodynamic studies were conducted non-GLP. The implications of this deficiency to the validity and significance of the safety data collected in the pivotal goat study are considered tolerable.

The mouse ECFA assay was originally central on the one hand in validating the potency assay, and on the other hand in correlating the potency data with the cartilage repair in clinically relevant setting, i.e. implantation to knee. Since a direct correlation between the potency and the cartilage repair in patients could not be demonstrated, the animal data would have been invaluable in providing evidence for this

interrelationship. Since the Applicant has later developed a new functional assay to follow potency of the Medicinal product during characterisation and process validation studies, the problems related to the validity of the ECFA assay in bridging the potency and clinical efficacy data is no more an issue.

The studies in goats are adequate to demonstrate proof of principle in a clinically relevant setting. However, the set of data is very limited and the Applicant did not demonstrate a valid correlation between the ECFA histology score and cartilage repair in the orthotopic goat model. In addition, although the orthotopic goat model demonstrates the proof of concept of ChondroCelect-like chondrocytes, this model is not fully representing the human situation (such as using membrane/ fibrin sealant) and is limited for cartilage repair in long-term.

Since data in humans are available, it is agreed that further data to obtain a real correlation between the ECFA histology score and cartilage repair in the goat model is not appropriate.

Regarding the fibrin sealants used together with ChondroCelect compatibility data for the fibrin glue TissuCol (Tisseel) have demonstrated the safe and effective use of this sealant with ChondroCelect in non-clinical studies. The concomitant use of Quixil in the pivotal clinical trial did not reveal any safety signal so far. The concomitant use of fibrin glue has been addressed in the SPC.

Efficacy

In one pivotal, multicentre, randomized, controlled phase III study ACI was compared to Microfracture with regards to structural repair and KOOS. Results of the histological analysis of structural repair at 12 months favour ChondroCelect and the difference is statistically significant for both qualitative and quantitative analysis. It was, however, acknowledged that this end point was not in compliance with GCP as it was developed during the conduct of the study as the original *a priori* determined primary efficacy point was considered as invalid.
The mean change in overall KOOS from baseline to the average of 12 to 18 months was slightly higher for patients in the ChondroCelect group than for patients in the microfracture group. The results fulfil the predefined criteria for non-inferiority and changes are clinically relevant.
The clinical data have also been analysed when all patients reached at least 36 months follow-up.
During the procedure the Applicant provided data for the pivotal clinical trial including graphical illustrations of a mixed model with time as a categorical variable. The additional mixed model analysis indicates that change (increase) from baseline in KOOS in the ChondroCelect group (CC) is numerically more pronounced when compared to the Microfracture group (MI), but does not give a statistically significant difference at months 36, 48 and 60 (when considering multiplicity) in favor of ChondroCelect. However, it does fulfill the formal requirement for non-inferiority. Neither BMI nor Gender has a significant influence on the overall KOOS. The results of time points beyond 36 months were to be taken only as descriptive, because of the small number of patients already reaching later follow-up time points (i.e. 48 and 60 months).

Safety

The overall safety summary shows that the main difference in treatment related adverse events compared to microfracture is related to the open knee surgery (arthrotomy) which causes an increase in joint swelling and possible joint effusion. Cartilage hypertrophy can be reduced by using a biomembrane to cover the lesion, and will therefore not pose a major safety concern in future applications of ChondroCelect. However, a higher number of patients in the microfracture arm have a treatment failure and require a subsequent surgical intervention. Therefore the short and long term complication rate is not higher for ChondroCelect compared to microfracture.
The Applicant has presented an acceptable RMP including a proposal for a confirmatory randomized controlled trial and an observational follow-up study.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

- User consultation

The user/readability testing is considered acceptable. The information on user testing provided by the Applicant was found to be satisfactory.

Risk-benefit assessment

The CAT, having considered the data submitted, was of the opinion that the risk management system should be requested according to the Article 14 (2) of the Regulation (EC) 1394/2007. There are the following particular causes for concern:

- There were deficiencies in the conduct of the pre-authorisation studies and uncertainties related to the result of the submitted single pivotal trial.
- There is unknown long-term durability of the product efficacy.
- Benefit/risk of the product is significantly influenced by the level of compliance with the defined procedures throughout the treatment with ChondroCelect, from the biopsy harvest till the correct physiotherapy.

Therefore, fully produced Risk Management Plan, including Pharmacovigilance plan, Risk Minimisation plan and Efficacy Follow-up plan was required. The details are described above in the Chapter 3.5 – Pharmacovigilance.

Benefits

The benefits of the ACI technique using ChondroCelect for smaller lesions (up to 5 cm2) are based on the demonstration of superiority for structural repair and non-inferiority in the clinical analysis (KOOS) compared to the standard treatment microfracture.

ACI techniques would particularly be suitable for larger defects ($\geq$4 cm^2), for which other suitable treatment does not exist. While limited data are available with ChondroCelect the literature data provided confirm this observation. The repair of full size chondrocyte defects and the restoration of functional cartilage need special consideration with the aim to reduce the risk of developing knee osteoarthritis on the long term.

Risks

The combination of current conservative and invasive therapies for cartilage injuries is associated with reasonably good control of symptoms and physical function in the short term. However, it is less clear whether these therapies provide a good long term outcome or whether the injury will have long term consequences, such as secondary arthrosis.

Balance

Given that microfracture is considered an effective standard treatment for femoral cartilage lesions below 3-4cm² size, and given the proven statistical non-inferiority of ACI with ChondroCelect to microfracture as well as the balanced overall safety profile the final overall B/R is considered positive. However, considering the limited data as efficacy is only based on one single pivotal trial, follow up of the clinical efficacy is required.

Recommendation

Based on the CAT/CHMP review of data on quality, safety and efficacy, the CAT considered by majority that the risk-benefit balance of ChondroCelect in the treatment of repair of single symptomatic cartilaginous defects of the femoral condyles of the knee (ICRS grade III or IV) in adults (Concomitant asymptomatic cartilage lesions (ICRS grade I or II) might be present) was favourable and therefore recommended the granting of the marketing authorisation.

The CAT, having considered the data submitted, was of the opinion that the risk management system should be requested according to the Article 14 (2) of the Regulation (EC) 1394/2007. There are the following particular causes for concern:

- There were deficiencies in the conduct of the pre-authorisation studies and uncertainties related to the result of the submitted single pivotal trial.
- There is unknown long-term durability of the product efficacy.
- Benefit/risk of the product is significantly influenced by the level of compliance with the defined procedures throughout the treatment with ChondroCelect, from the biopsy harvest till the correct physiotherapy.

The CHMP agreed with the Benefit-Risk assessment and recommendation for approval of the Marketing Authorisation for ChondroCelect as expressed by the CAT.

The CHMP had a comment to amend the wording of the indication (section 4.1 of the SPC) as follows:

Repair of single symptomatic cartilage defects of the femoral condyle of the knee (International Cartilage Repair Society [ICRS] grade III or IV) ~~from 2 cm² onwards~~ in adults. Concomitant asymptomatic cartilage lesions (ICRS grade I or II) might be present
Demonstration of efficacy is based on a randomised controlled trial evaluating the efficacy of ChondroCelect in patients with lesions between 1-5cm^2.

The scientific reasons for this change are described below:

CHMP considered that putting a precise lower boundary would prevent physicians from treating patients on-label who have lesions in sizes formally below 2cm^2, for whom, however, ChondroCelect treatment might nevertheless be medically indicated by the individual decision of the treating physician. Likewise, CHMP recognised the greater need for the treatment of larger lesions, but was concerned about the limited amount of data available for larger lesions. Therefore, in line with previous similar scenarios, the CHMP has taken the approach to put an indication that is permissive, but to point out the limitations of the data and to clearly inform the prescribing physician in section 4.1. A wording like this is usually perceived as stronger as compared to a wording in section 5.1, thus being more in line with the overall outcome of the CAT draft opinion. This allows the physician to take an informed decision for a particular patient's situation based on the knowledge of the availability of evidence from the pivotal study.