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分担研究報告書

再生医療等製品（安全性等の評価方法）に関する国際標準化

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研究要旨

細胞加工製品の臨床応用における品質・安全性上の課題として造腫瘍性が挙げられ、特にiPS 細胞加工製品において適切に評価される必要があり、試験法の検証が速やかになされることが重要である。細胞加工製品の実用化促進が期待される国際展開においては、検証された造腫瘍性評価法の標準プロトコールの国際的な共有が強く望まれるが、造腫瘍性評価の国際標準化や国際規制調和は未だなく、国際ガイドラインも未発出である。本研究は、臨床応用が進む iPS 細胞加工製品について、品質・安全性確保上の大きな課題である造腫瘍性とゲノム不安定性の評価法の開発・検証を促進し、先行主導的に国際標準を獲得することで、再生医療等製品分野での薬事規制における国際調和を促し、日本企業が開発する iPS 細胞加工製品の国際競争優位を獲得することを目的とする。国際標準化を目指した活動として、以下の研究を実施した。①in vitro 造腫瘍性試験法の国際標準プロトコールの確立および多施設検証については、国立医薬品食品衛生研究所（NIHS）と再生医療イノベーションフォーラムの多能性幹細胞安全性評価委員会（FIRM-CoNCEPT）が協働して、浮遊培養細胞の IL-2 非依存的細胞増殖特性解析試験法の検討を行った。標準プロトコールを作成し、参加4 施設において、0.01%の割合で正常 T 細胞に混在する形質転換細胞株 MOLT-4 の検出を、28 日間の培養で達成した。②標準陽性対照細胞に資するゲノム不安定性誘導 iPS 細胞株作製については、ゲノムに挿入するプラスミドのデザインとその iPS 細胞への導入を行い、薬剤に応答してがん抑制遺伝子 TP53 を CRISPR/Cas9 で切断する iPS 細胞のクローンを得た。③官民での国際競争戦略と知財・標準化戦略の検討では、iPS 細胞加工製品の各国の規制と標準化の動向に関する調査研究を行い、総説論文を作成した。iPS 細胞加工製品の造腫瘍性試験法の国際標準化の実現には、国際プラットホームで試験法のテーマ化を日本として提案し、国際合意を得ることが鍵になる。国際的認知を得るには、議論の深化に必要な科学的なエビデンスが不可欠であり、本研究の成果は、細胞加工製品の造腫瘍性評価およびゲノム不安定性評価の国際コンセンサスの熟成に資するものであり、国際標準化の促進が期待さ

れる。

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Ａ．研究目的

細胞加工製品の臨床応用における品質・安全性上の課題として造腫瘍性が挙げられ、特に iPS 細胞加工製品において適切に評価される必要があり、試験法の検証が速やかになされることが重要である。細胞加工製品の実用化促進が期待される国際展開においては、検証された造腫瘍性評価法の標準プロトコールの国際的な共有が強く望まれるが、造腫瘍性評価の国際標準化や国際規制調和は未だなく、国際ガイドラインも未発出である。日本では、iPS 細胞加工製品の造腫瘍性評価について、評価法の確立と国際標準化を迅速に図るべく、世界に先駆けて国内で展開され、留意点がいち早くガイドライン化・共有されている。国立医薬品食品衛生研究所（国立衛研）と再生医療イノベーションフォーラム（FIRM）の多能性幹細胞安全性評価委員会（FIRM-CoNCEPT）では、AMED 研究課題において iPS 細胞由来製品の造腫瘍性評価に係る多施設検証（MEASURE プロジェクト）を協働して実施しており、Health and Environmental Sciences Institute（HESI）の細胞治療委員会（CT-TRACS）の国際コンソーシアム活動にも参加している。しかしながら、iPS 細胞加工製品の造腫瘍性に関する種々の評価法の能力と限界に関する検証と国際的認知・科学的議論がまだ不足しており、最終目標とする ISO や ICH の国際プラットフォームでの具体的な提案や議論に至っていない。さらに iPS 細胞加工製品の造腫瘍性について考慮すべき点として、腫瘍形成を惹起するハザードであるゲノム不安定性があるが、その評価に際して、汎用性・安定性・品質管理の要求基準を満たす陽性対照細胞が存在しないことから、製品の腫瘍形成との関連性における科学的エビデンスが不足している。本研究は、臨床応用が進む iPS 細胞加工製品について、品質・安全性確保上の大きな課題である造腫瘍性とゲノム不安定性の評価法の開発・検証を促進し、先行主

導的に国際標準を獲得することで、再生医療等製品分野での薬事規制における国際調和を促し、日本企業が開発する iPS 細胞加工製品の国際競争優位を獲得することを目的とする。さらに、再生医療等製品のプラットホーム技術の国際標準化と国際標準の活用により、国際市場展開を行う技術の知財確保を併せた知財・標準化戦略を産学官連携体制で推進することを目指す。

Ｂ．研究方法

iPS 細胞加工製品の造腫瘍性試験法の国際標準化の実現には、ISO や ICH といった国際プラットホームで、試験法のテーマ化を日本として提案し、国際合意を得ることが鍵になる。国際的認知を得るには、議論の深化に必要な科学的なエビデンスが不可欠であるため、①標準プロトコールに沿って in vitro 造腫瘍性試験法の多施設検証をまず実施し、評価方法の能力と限界を示した。同時に、造腫瘍性との関連が深いゲノム不安定性試験法について、②試験法としての妥当性を判断する陽性対照細胞の作製を開始した。また、③造腫瘍性およびゲノム不安定性評価についての国際標準化戦略に向けた調査研究も実施した。

B-1 in vitro 造腫瘍性試験法の国際標準プロトコールの確立および多施設検証

T 細胞は浮遊細胞であるため、混在する形質転換細胞を検出する際に、足場非依存的に増殖する悪性形質転換細胞を検出する軟寒天コロニー形成試験法は適用できない。したがって、不死化細胞を検出する際に用いる細胞増殖特性解析法を適用することになる。HTLV-1 に感染した T 細胞に形質転換が生じるとインターロイキン 2（IL-2）非依存的な増殖を示すようになることが報告されている（Science 1995;269(5220):79-81.）。このことから T 細胞の IL-2 非依存的な増殖特性が、形質転換細胞を検出する一つの指標になると考えられている（図 1)。この IL-2 非依存的増殖をモニターする試験法は、開発現場で CAR-T 細胞製品の非臨床試験として既に利用されているが、陽性対照細胞の同定とそのスパイク試験は実施されていないことから、検出感度や再現性といった試験法の性能は確認されていない。標準化においては、試験法の性能を確認する多施設検証が必要であるため、FIRM-CoNCEPT の協力のもと、Axcelead Drug Discovery Partners 株式会社、株式会社ボゾリサーチセンター、シミック株式会社および株式会社 Ig-M の 4 施設において、T 細胞の IL-2 非依存的細胞増殖特性解析試験を実施した。試験の概要を、図 2 に示す。作成した『浮遊培養細胞の増殖特性解析に関する手順書』は、資料として添付した。

B-1-1 IL-2 非依存的細胞増殖特性解析試験法

ヒト末梢血単核細胞（Lonza）から EasySep Human T cell Isolation Kit （ StemCell Technologies）で T 細胞を精製した後、3 日間 CD3/CD28 ビーズ（StemCell Technologies）で活性化した。マグネットビーズを除去し

た後、1×10^6個のT細胞に10個 (0.001%)、100個 (0.01%)、1000個 (0.1%)または10000個 (1%)のMOLT-4細胞（ATCC）をスパイクした（3日目）。これらの細胞をIL-2非存在下で培養を開始し、5日目に2倍希釈した。7日目に10倍希釈し、21日目（多施設検証1回目）または28日目（多施設検証2回目）まで培養した。継時的に細胞計数を行い、3、5、7、10、14、17、21、24および28日目の生細胞数をグラフ化し、増殖曲線を作成した。統計解析は、生細胞数を対数変換し、two way repeated measures ANOVAの後、post-hocテストとしてDunnett's Methodを行い、$p < 0.05$を有意差ありとした。

B-2 標準陽性対照細胞に資するゲノム不安定性誘導 iPS 細胞株作製

ゲノム不安定性試験の標準陽性対照細胞として、コンディショナルにゲノム編集を起こして、がん関連遺伝子に変異が導入される iPS 細胞株を作製した (図 3)。薬剤等でがん関連遺伝子に適時に変異を誘導するため、長期の培養・継代による変異蓄積の影響を除外でき、培養期間や継代数が異なっていても、施設間で品質の揃った細胞の使用が可能になる。ゲノム不安定性誘導 iPS 細胞株の作製に用いる細胞は、一般的に入手可能で臨床株と同様な方法で樹立された研究用 iPS 細胞株から選択した。また変異を導入するがん関連遺伝子としては、がん組織で最も変異が報告されているがん抑制遺伝子 TP53 を採用した。ゲノム不安定性誘導 iPS 細胞株の作製は、株式会社 GenAhead Bio で行った。

B-2-1 Single Guide RNA 発現プラスミドの調製

AAVS1 site へのゲノム編集報告例 (Cell Stem Cell 2014;15(2):215-226.) より、Single Guide RNA (sgRNA) の標的配列として以下のものとした。

AAVS1-T2: TGACTGCTTGTAGATGGCCA

上記配列をもとにオリゴ DNA を化学合成し、U6 Promotor の下流にライゲーションすることにより sgRNA 発現プラスミドを作製した。

B-2-2 ドナープラスミドの作製

Inducible-Cas9 発現カセット及び Tet-on 調節因子発現カセットを挿入配列とするドナープラスミドを、図 4 (a), (b) の通り作製した。Inducible-Cas9 発現カセット挿入ドナープラスミド (TRE-Cas9/sgRNA donor) は、図 4 (a) の構造を、(1) 上流 homology arm (HA1) 断片、(2) splicing acceptor (SA) 及び T2A peptide による puromycin-*N*-acetyltransferase (PuroR) 発現カセット、(3) U6 promoter 制御の sgRNA 発現カセット、(4) inducible (TRE3G promoter)-Cas9 発現カセット、(5) 下流 homologyarm (HA2) 断片に分けて調製した。(1), (5) の HA 断片は、ヒト iPSC 1231A3 (hiPSC-A3) より Tissue Genomic DNA Extraction Mini Kit (FAVORGEN) を用いて調製したゲノム DNA を鋳型に、sgRNA の上流配列、下流配列 (各約 1,500 bp) を増幅して調製した。(2)

PuroR 発現カセットは、SA-T2A を PuroR の上流に配置したプライマーを用い、PuroR 発現プラスミドを鋳型とした PCR 増幅により調製した。(3) U6-sgRNA 発現カセットは、U6 promoter と tracr 配列間に TP53 遺伝子を標的とする sgRNA 配列 (TP53-R175H-S1534, 5'-AGCACATGACGGAGGTTGTG-3') を挿入したプラスミドを人工合成し、PCR 増幅により調製した。(4) TRE3G-Cas9 発現カセットは、国立医薬品食品衛生研究所から提供された pTRE3G vector (TaKaRa Bio) に Cas9 遺伝子を挿入し、TRE3G-Cas9 発現プラスミドを鋳型とした PCR 増幅により調製した。TRE-Cas9/sgRNAdonor は (1) から (5) を NEBuilder HiFi DNA Assembly キット (New England BioLabs) を用いて図 4 (a) の通り Gibson assembly して作製した。TRE-Cas9 donor は、(3) U6-sgRNA 発現カセットを除くフラグメントを TRE-Cas9/sgRNA donor と同様にアセンブルして作製した。

Tet-on 調節因子発現カセットを挿入配列とするドナープラスミド (Tet3G donor) は、図 4 (b) の構造を、(6) 上流 homology arm (HA1) 断片、(7) splicing acceptor (SA) 及び T2A peptide による aminoglycoside 3′-phosphotransferase (NeoR) 発現カセット、(8) Tet-on 調節因子 (Tet3G) 発現カセット、(9) 下流 homology arm (HA2) 断片に分けて調製した。(6), (9) の HA 断片は、hiPSC-A3 より Tissue Genomic DNA Extraction Mini Kit を用いて調製したゲノム DNA を鋳型に、sgRNA の上流配列、下流配列 (各約 1,500 bp) を増幅して調製した。(7) NeoR 発現カセットは、SA-T2A を NeoR の上流に配置したプライマーを用い、NeoR 発現プラスミドを鋳型とした PCR 増幅により調製した。(8) Tet3G 発現カセットはまず、国立医薬品食品衛生研究所から提供された pCMV-Tet3G plasmid (TaKaRa Bio) を鋳型に Tet3G 及び rBGpA シグナル配列を PCR 増幅し、pCAG vector に NEBuilder HiFi DNA Assembly キットを用いて挿入して CAG-Tet3G 発現プラスミドを作製した。CAG-Tet3G 発現プラスミドを Sal I 制限酵素 (New England BioLabs) 及び AgeI 制限酵素 (New England BioLabs) で消化後、アガロースゲル電気泳動により目的断片の大きさのフラグメントを CAG-Tet3G 発現カセットとして調製した。Tet3G donor は、(6) から (9) の NEBuilder HiFi DNA Assembly キットを用いて図 1 (b) の通り Gibson assembly して作製した。

B-2-3 ドナープラスミドの作製

hiPSC-A3 及びヒト iPSC 1383D6 (hiPSC-D6) への transfection は、それぞれ表 1 及び表 2 に示す条件で、2. の sgRNA 発現プラスミド、Streptococcus pyogenes Cas9 発現プラスミド、3. の各ドナープラスミド、及び puromycin-*N*-acetyltransferase 発現プラスミドと共に NEPA21 (Nepagene) を用いて transfection した。transfection 後の細胞は、HDR Enhancer (Integrated DNA Technologies) を終濃度 0、又は 0.5 μg/mL で含む培地で 24 h 培養した。その後、表 1 及び表 2 の条件

に従い、puromycin を終濃度 0、0.35、0.6、又は 1.0 μg/mL で培養培地に添加した。

B-3 官民での国際競争戦略と知財・標準化戦略の検討

海外のステークホルダーとのコンセンサスの熟成を促すため、日米 EU の ICH3 極の薬事規制と標準化プラットホームの動向について情報共有を行う必要がある。官民での国際競争戦略と知財・標準化戦略の検討に資する研究として、iPS 細胞加工製品の各国の規制と標準化の動向に関する調査研究を行った。

（倫理面への配慮）

ヒト由来の生体試料を用いる場合は、国立医薬品食品衛生研究所「研究倫理審査委員会規程」を遵守した上で研究を実施した。

C．研究結果

C-1 in vitro 造腫瘍性試験法の国際標準プロトコールの確立および多施設検証

本研究は、浮遊培養細胞である T 細胞に混在する形質転換細胞を、IL-2 非存在下で培養することによって検出する試験法の標準プロトコールの作成と感度を多施設で検証することを目的とする。ヒト末梢血単核細胞から単離した T 細胞を活性化し、1×10^6 個の T 細胞に、MOLT-4 細胞を 1, 0.1, 0.01 または 0.001%スパイクした。IL-2 非存在下で 21 日目まで細胞数を計測し、細胞数の継時変化を観察した（図 5）。また正常 T 細胞の IL-2 依存的増殖を確認するため、IL-2 存在化で培養するスパイクなしの正常 T 細胞サンプルも設けた。IL-2 存在下では、T 細胞は 14 日目まで増殖を示し、その後に緩やかな減少に転じた。また全 4 施設で同様な結果が得られることを確認できた。一方、IL-2 非存在下では、7 日目まで IL-2 存在下と同様な増殖を示したが、それ以降生細胞数が著しく減少した。この減少は、IL-2 非存在下の培養による正常 T 細胞の死滅によるものである。増殖曲線のパターンは全施設で同様な結果が観察され、スパイクなしのサンプルと比較して、概ね 17 日目に 1%スパイクサンプルでの細胞数の有意な差が見られ、21 日目に 0.1%スパイクサンプルでの細胞数の有意な差が見られた。これらの結果は、施設の違いにも関わらず、21 日目までの観察で正常 T 細胞に 0.1%割合でのスパイクした MOLT-4 細胞が検出できることを示唆している。しかしながら、21 日目まで培養を行っても、0.01%スパイクサンプルの立ち上がりは、全施設において見られなかった。21 日間の培養では 0.01%スパイクサンプルの検出が難しいと判断されたため、2 回目の多施設検証試験では、標準プロトコールを改訂し、培養期間を延長して、24 日目と 28 日目の細胞計数を含めての検討を行った（図 6）。2 回目の多施設検証においても、21 日目までの観察では同様な結果が得られた。IL-2 存在下では、T 細胞は 14 日目まで増殖を示し、その後に緩やかな減少に転じた。一方、IL-2 非存在下では、生細胞は 7 日目以降著しく減少し、28 日目では生存率数%もしくは検出

できないレベルまで下がった。また、概ね17日目に1%スパイクサンプルでの生細胞数の立ち上がりが見られ、21日目に0.1%スパイクサンプルでの生細胞数の立ち上がりが見られた。1回目の多施設検証で立ち上がりが認められなかった0.01%スパイクサンプルは、培養期間を延長することにより増加が観察された。スパイクなしに比べて、施設Aにおいては28日目に、施設B、CおよびDにおいては、24と28日目に有意な差が確認できた。全施設で正常T細胞に混在する形質転換細胞（MOLT-4）を0.01%の高感度で検出する標準プロトコールを確立した。

C-2 標準陽性対照細胞に資するゲノム不安定性誘導 iPS 細胞株作製

先行実施した Tf No. 1-1 から 1-4 の puromycin 処理後の生存細胞は乏しく、それぞれの生存コロニー数を表3に示す。生存コロニーに乏しい場合、ドナープラスミド配列の挿入が検出された細胞群からは、少数のピックアップにより配列挿入株の取得が見込まれるため、これらの細胞群から親株配列と挿入ドナープラスミド配列とのつなぎ目領域を増幅する PCR (junction PCR) により配列挿入株が含まれる細胞群を選択することとした (図 7A)。

各 PCR 増幅産物の電気泳動像を図 7B に示す。 hiPSC-D6 の Tf No. 1-2 及び 1-1 の細胞群でドナープラスミド配列の挿入で想定されるサイズの増幅産物が検出され、これらの細胞群に配列挿入株が含まれることが示唆された。

C-3 官民での国際競争戦略と知財・標準化戦略の検討

日米 EU の ICH3 極における多能性幹細胞由来製品に関する規制の概要と、その標準化に取り組んでいる代表的なコンソーシアムについて紹介した。本成果は、総説論文 “Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells.”のタイトル名で、国際科学雑誌 Stem Cell Reports に受理され、出版中である。要旨の日本語は以下の通りである。

『現在，胚性幹細胞や人工多能性幹細胞などの多能性幹細胞（PSC）を由来とする細胞治療薬（CTP）が多くの国で開発されており，一部は臨床試験段階にある。CTP は、国や地域によって分類が異なる。また、有効性・安全性・品質の評価も、従来の低分子医薬品やバイオ医薬品とは異なり、生きた細胞の複雑な特性やアンメットメディカルニーズを反映している。PSC 由来品を含む CTP の評価については、国際的なガイドラインがないため、国や地域による関連法規の違いに注意する必要がある。PSC 由来 CTP の開発・世界流通を促進するために，評価手法や規制の標準化・調和を図る国際コンソーシアムが組織され，活発に活動している。本稿では，米国，欧州連合（および英国），日本における PSC 由来 CTP の関連法規の概要と，その標準化に取り組んでいる代表的なコンソーシアムについて紹

介する。』

D．考察

再生医療のような先端的な医療モダリティについては、安全性や品質の確保のための個別の評価技術の国際標準化を推進することが、規制当局の評価・意思決定の効率化・迅速化に有効である。国際標準化により iPS 細胞加工製品の造腫瘍性およびゲノム不安定性評価法の妥当性が認められ、当該評価法を用いた製品の評価が国内外の規制当局に受け入れられることで、承認申請に要する日本企業の負担軽減に寄与するとともに、製品の国際市場での迅速な普及展開を図ることができる。すなわち日本での試験方法・運用等を国際標準化することで、主要国の規制当局の審査に関する国際規制調和が促進され、日本で実施した試験と評価がそのまま海外での承認審査でも活用され易くなり、国際ビジネス展開の環境整備が進む。また国際標準化を行う造腫瘍性評価については、これまでの多施設検証に参加した日本の製薬企業・CRO で試験方法に関するトレーニングと技術移転が進展し、製品の品質試験・非臨床試験でのデータ取得体制が国内で既に整備されている。国際標準化された試験方法として海外の規制当局に認知・受容されれば、海外での治験届・製造販売承認申請時の迅速な審査のみならず海外企業から国内 CRO への試験委託増加が期待される。

E. 結論

iPS 細胞加工製品の造腫瘍性試験法が国際的認知を得るには、議論の深化に必要な科学的なエビデンスが不可欠である。本研究では、in vitro 造腫瘍性細胞検出試験法の標準プロトコールに沿った多施設検証データを取得して、試験法の頑健性を確認した。造腫瘍性のハザードであるゲノム不安定性を評価する試験法については、その妥当性判断に用いる標準陽性対照細胞の作製に取り組み、薬剤誘導ゲノム不安定性 iPS 細胞株のクローンを得た。iPS 細胞加工製品の各国の規制と標準化の動向に関しては、調査研究を行って総説論文を発表した。本研究成果は、細胞加工製品の造腫瘍性評価およびゲノム不安定性評価の国際コンセンサスの熟成に資するものであり、国際標準化の促進が強く期待される。

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Ｆ．研究危険情報

なし

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Ｈ．知的財産権の出願・登録状況

H-1. 取得特許

なし

H-2. 実用新案登録

なし

H-3. その他

なし

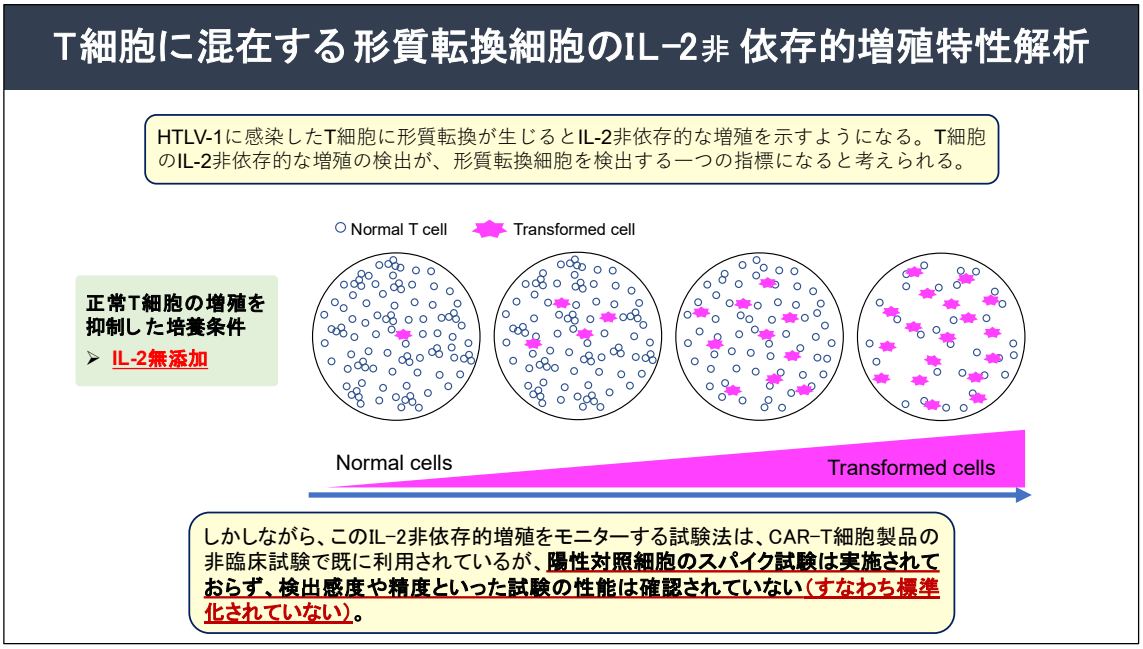


図 1　T 細胞に混在する形質転換細胞の IL-2 非依存的増殖特性解析

Day 0　（1）PBMCを解凍　　（2）PBMCから T細胞を分離　　（3）CD3/CD28ビーズで T細胞の活性化

凍結バイアル
5.0 ×10^{7}個以上

PBMC

2 ×10^{7}個以上
T細胞を回収

75 cm^2 フラスコ ×2
1フラスコあたり1～2 ×10^{7}個

Day 3　（4）増幅した活性化T細胞（ 約2～3倍）を回収し、CD3/CD28ビーズを除去する。

（5）6ウェルプレート の1ウェルあたり 活性化T細胞を1.0 ×10^{6}個、18ウェル分播種。

（ 活性化T細胞の濃度: 5.0 ×10^{5}個/mL）

（6）6ウェルプレート の1ウェルあたりMOLT-4を以下のとおりスパイクする。

① Spike 0　IL-2（+）
② Spike 0　IL-2（-）
③ Spike 1x10^{1}個
④ Spike 1x10^{2}個
⑤ Spike 1x10^{3}個
⑥ Spike 1x10^{4}個

1頁

Day 5　（7）新しい6ウェルプレート を3枚準備する。

（8）ウェルに細胞懸濁液とX-VIVO 10培地を等量加え、2倍希釈する。

Day 7　（9）新しい75 cm^{2}フラスコを18個準備する。

（10）75 cm^{2}フラスコに、細胞懸濁液: X-VIVO 10培地＝1： 9になるよう 加え、10倍希釈する。

① Spike 0 IL-2（+）　② Spike 0 IL-2（-）　③ Spike 1x10^{1}個　④ Spike 1x10^{2}個　⑤ Spike 1x10^{3}個　⑥ Spike 1x10^{4}個

＊ 以降は、細胞数カウント のみ実施
（ Days 10, 12, 14, 17, 21, 24, 28）

2頁

図 2　浮遊培養細胞の IL-2 非依存的増殖特性解析試験法プロトコールの概要

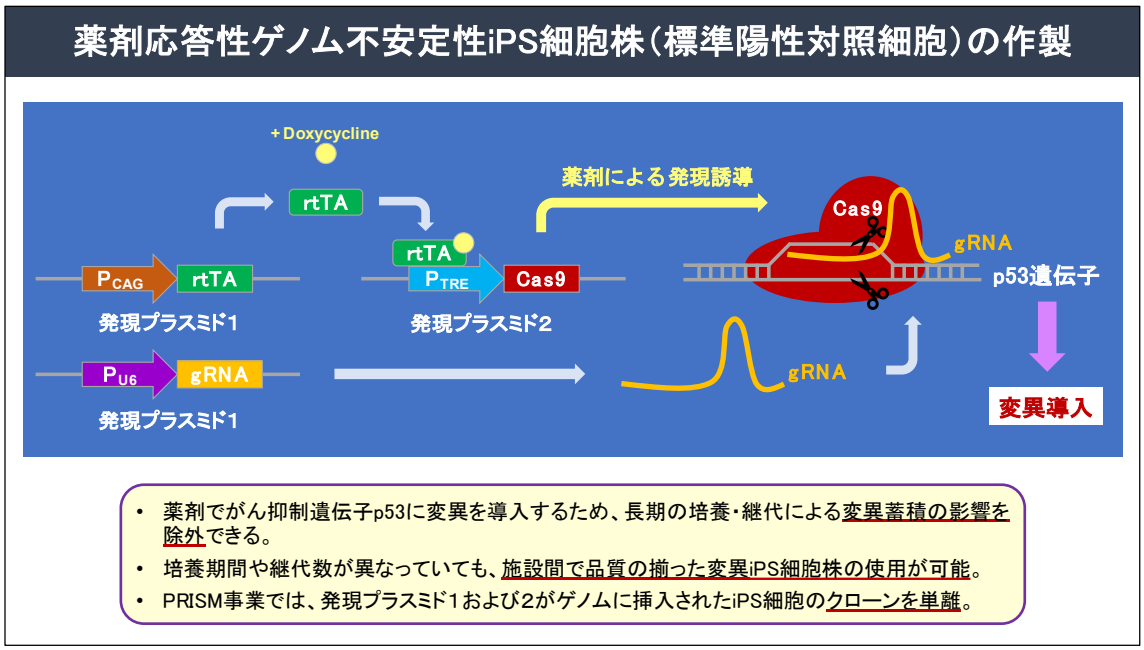


図 3　薬剤応答性ゲノム不安定性 iPS 細胞株の概要

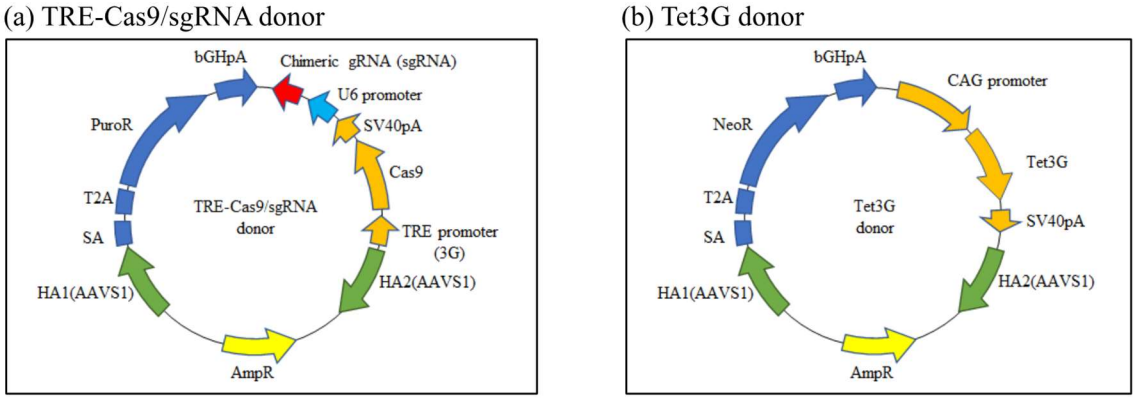


図 4　ドナープラスミドマップ

表 1　hiPSC-A3 への Transfection 条件

Transfection No. (Tf No.)	1-1	1-2	1-3	1-4
パルス条件	150 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor			
HDE Enhancer	0.5 μM			
Puromycin 濃度 [μg/mL]	0.2	0.35	0.6	1.0

Transfection No. (Tf No.)	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8
パルス条件	125 V, 5 msec, 2 pulses				150 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor		TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM		0.5 μM		0 μM		0.5 μM	
Puromycin 濃度 [μg/mL]	0.2							

Transfection No. (Tf No.)	2-9	2-10	2-11	2-12
パルス条件	175 V, 2.5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM		0.5 μM	
Puromycin 濃度 [μg/mL]	0.2			

Transfection No. (Tf No.)	2-13	2-14	2-15	2-16	2-17	2-18	2-19	2-20
パルス条件	125 V, 5 msec, 2 pulses				150 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor		TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM		0.5 μM		0 μM		0.5 μM	
Puromycin 濃度 [μg/mL]	0.35							

Transfection No. (Tf No.)	2-21	2-22	2-23	2-24	2-25	2-26	2-27	2-28
パルス条件	175 V, 2.5 msec, 2 pulses				125 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor		TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM		0.5 μM		0 μM		0.5 μM	

Puromycin 濃度 [μg/mL]	0.35	0.6

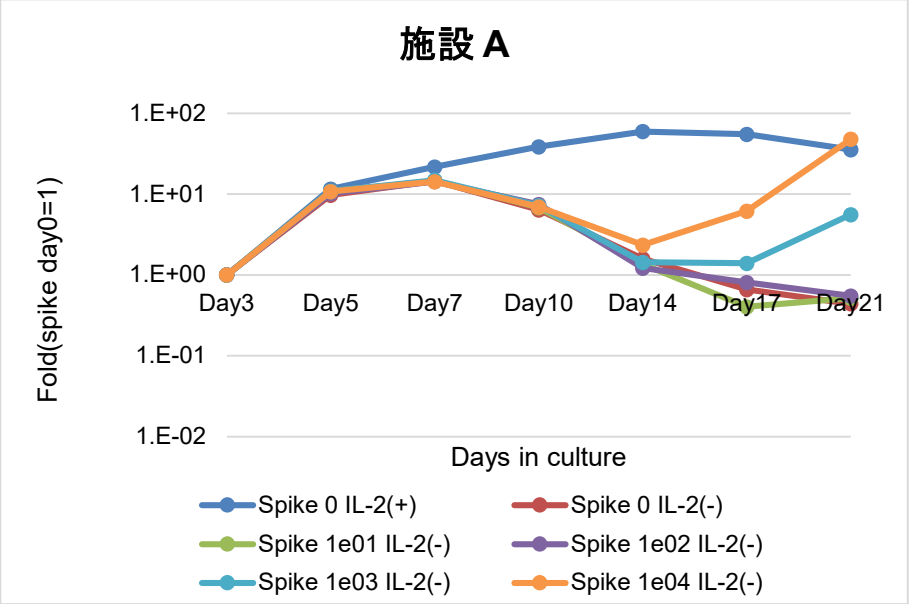
Transfection No. (Tf No.)	2-29	2-30	2-31	2-32	2-33	2-34	2-35	2-36
パルス条件	150 V, 5 msec, 2 pulses				175 V, 2.5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor		TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM		0.5 μM		0 μM		0.5 μM	
Puromycin 濃度 [μg/mL]	0.6				0.6			

表 2　hiPSC-D6 への Transfection 条件

Transfection No. (Tf No.)	1-1	1-2	1-3	1-4
パルス条件	150 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor			
HDE Enhancer	0.5 μM			
Puromycin 濃度 [μg/mL]	0.2	0.35	0.6	1.0

Transfection No. (Tf No.)	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8
パルス条件	150 V, 5 msec, 2 pulses							
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor		TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM	0.5 μM	0 μM	0.5 μM	0 μM	0.5 μM	0 μM	0.5 μM
Puromycin 濃度 [μg/mL]	0.2				0.35			

Transfection No. (Tf No.)	2-9	2-10	2-11	2-12
パルス条件	150 V, 5 msec, 2 pulses			
ドナープラスミド	TRE-Cas9 donor, Tet3G donor		TRE-Cas9/sgRNA donor, Tet3G donor	
HDE Enhancer	0 μM	0.5 μM	0 μM	0.5 μM
Puromycin 濃度 [μg/mL]	0.6			



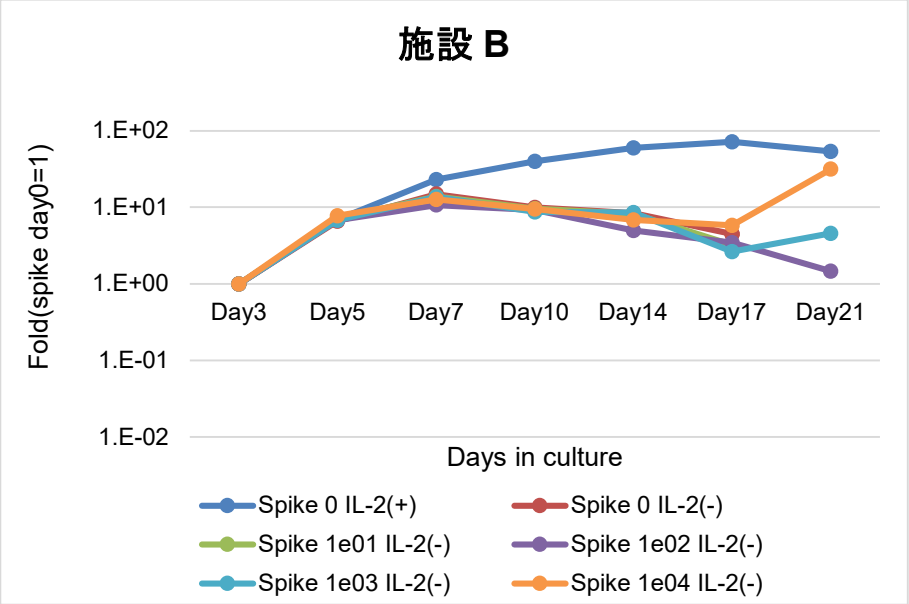
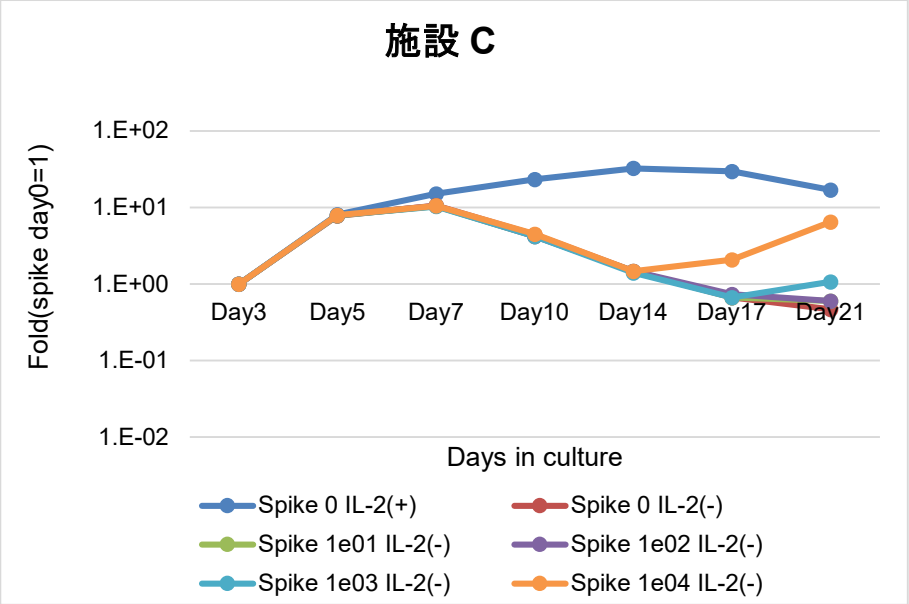


図 5　IL-2 非依存的増殖特性解析の多施設検証 1 回目



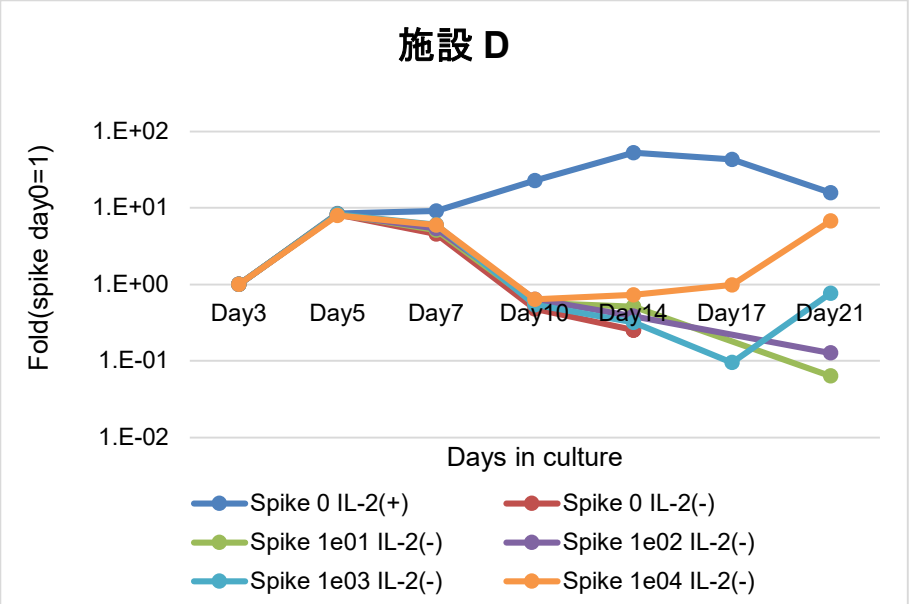


図 5　IL-2 非依存的増殖特性解析の多施設検証 1 回目（続き）

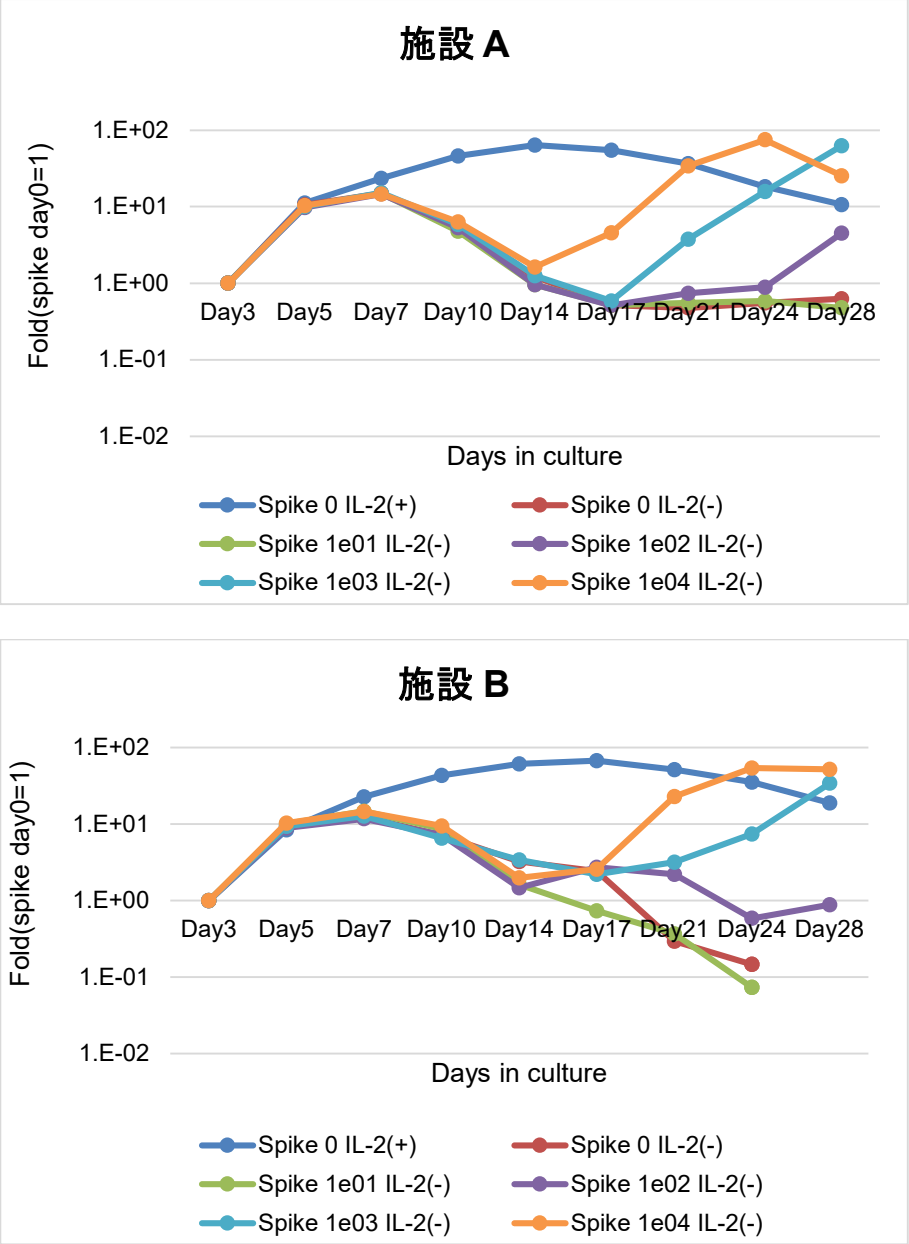
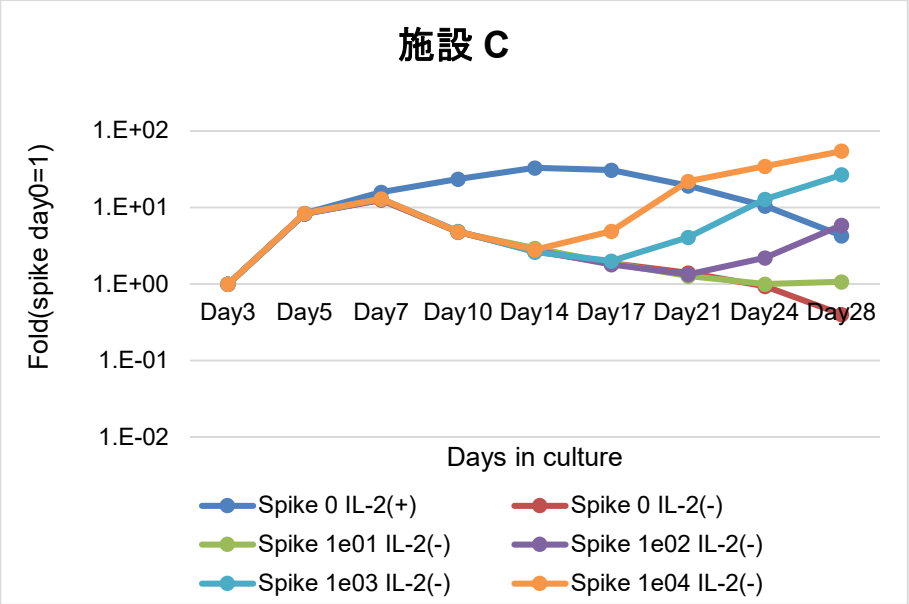


図 6　IL-2 非依存的増殖特性解析の多施設検証 2 回目



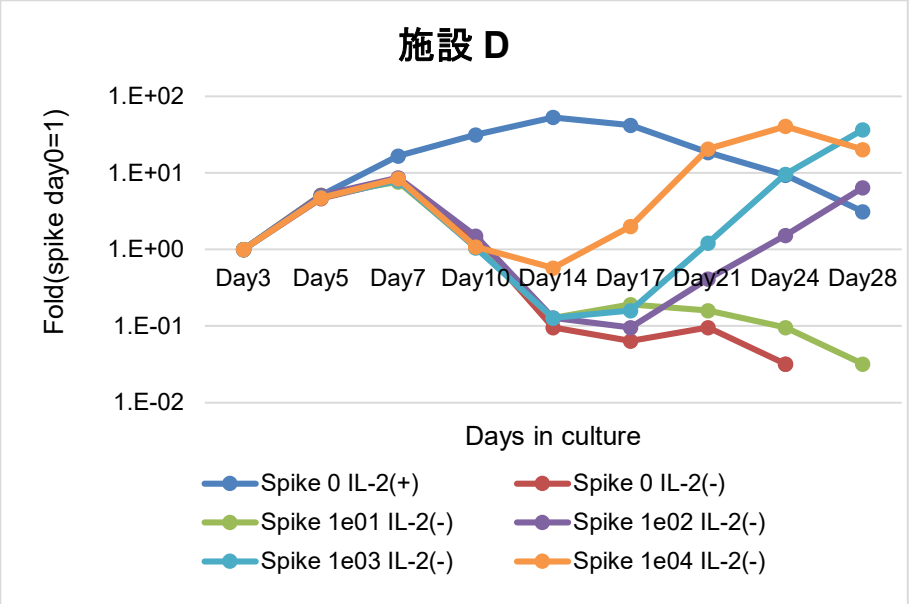


図 6　IL-2 非依存的増殖特性解析の多施設検証 2 回目（続き）

表 3　Tf No.1-1 から 1-4 の Puromycin 処理後の生存コロニー数

	Tf No. 1-1	Tf No. 1-2	Tf No. 1-3	Tf No. 1-4
hiPSC-A3	<50	<5	0	0
hiPSC-D6	<50	<5	<5	<5

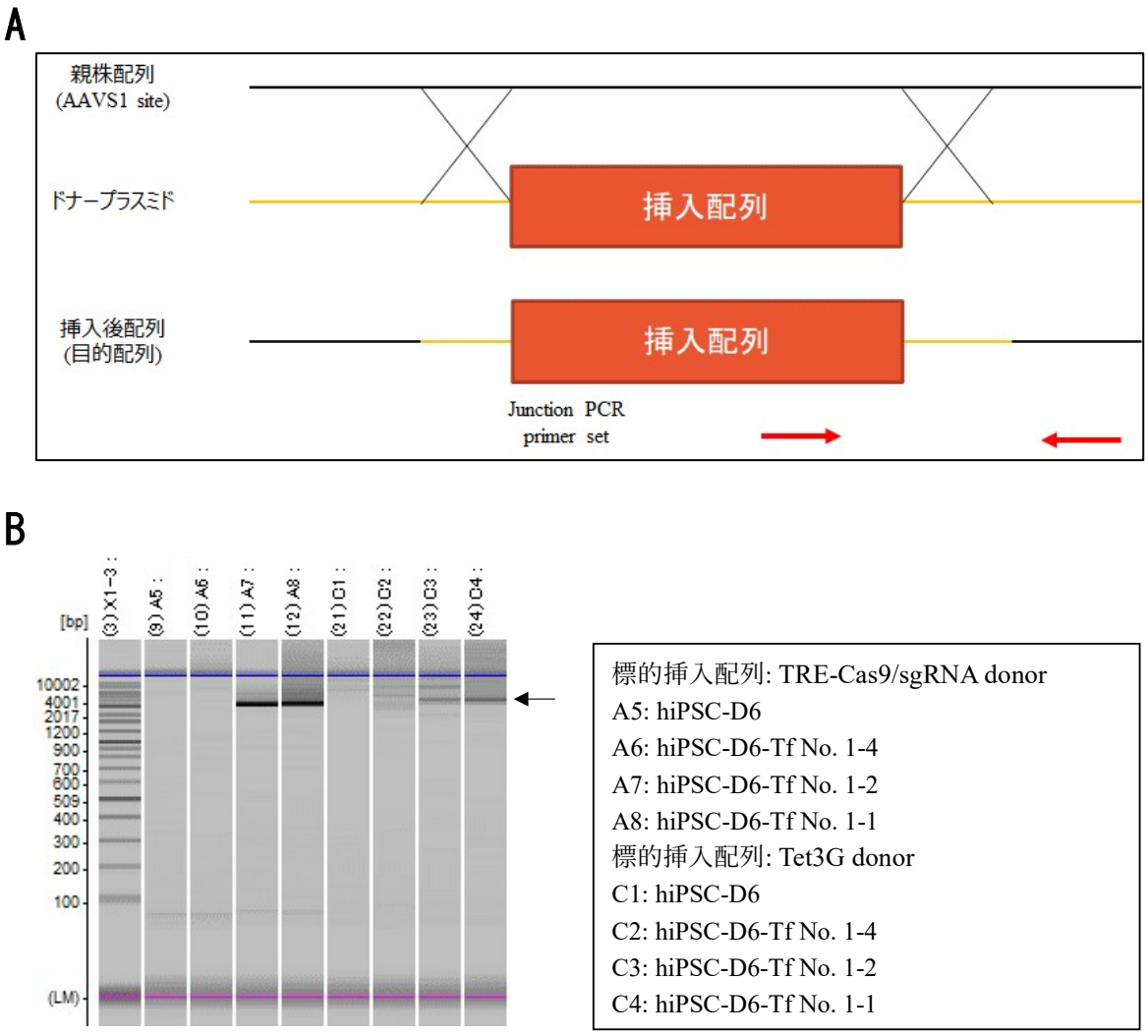


図 7　Junction PCR の結果 (A) Junction PCR の標的配列。(B) Junction PCR の増幅産物の電気泳動像（矢印：ドナープラスミド配列の挿入で想定されるサイズ）。

浮遊培養細胞の
増殖特性解析に関する手順書

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国立医薬品食品衛生研究所
再生・細胞医療製品部

浮遊培養細胞の増殖特性解析に関する手順書

改訂履歴表

版番号及び 改訂番号	年月日	改訂内容	改訂理由
1	2023/01/25	制定	
2	2023/01/30	修正	rIL-2 製品の明確化、記載整備
3	2023/02/28	修正	培養期間の延長

1. 目的

CAR-T 療法による細胞加工製品の安全性を評価するには、T 細胞に遺伝子導入したことによる腫瘍形成又はがん化の可能性を考慮する必要があり、正常 T 細胞は IL-2 依存的に増殖、形質転換細胞は IL-2 非依存的に増殖するという特性を用いた「IL-2 非依存的増殖試験」を提唱しているところである。

本手順書の工程を標準プロトコルとして多施設で実施し、施設間における本試験法の検出限界、特異性、精度、陽性対照のあり方、結果の再現性などについて比較及び検証するため、本手順書は浮遊培養細胞の増殖特性解析に関する手順を定める。

2. 適用範囲

本手順書は、細胞加工製品の品質・安全性を評価するため、国立医薬品食品衛生研究所から委託試験を依頼された研究機関（以下「委託試験実施機関」という。）が本手順書を用いて実施する場合において、試験に用いる原料、資材、並びに機器類などを含む一連の手順に適用する。

3. 責任体制

国立医薬品食品衛生研究所と委託試験実施機関との間で予め取り決めた手順（記録、並びにその記録の保管を含む。）に従い、本試験の特性を把握した上で実施する。

4. 遵守事項

労働安全衛生法

世界保健機構　実験室バイオセーフティマニュアル

5. 留意事項

5-1　共通の原料及び資材

試験に係る以下の原料及び資材について、メーカーと型番は指定であるが、指定外の原料、資材並びに機器類に関しては代替可である。なお、委託試験実施機関は使用したメーカーと型番は、国立医薬品食品衛生研究所へ報告するものとする。

品名	型番	メーカー	保管温度
ヒト末梢血 PBMC	CC-2702	Lonza	液体窒素中
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	4℃
FBS（非働化済のもの）	172012	Sigma®	-20℃以下
DNase I	2270A	Takara Bio	-20℃以下
EasySep Buffer	20144	STEMCELL Technologies	4℃
EasySep Human T cell Isolation Kit	17951	STEMCELL Technologies	4℃
Dynabeads Human T-Activator CD3/CD28	11131D	Thermo Fisher	4℃
BSA	A2934	Sigma-Aldrich	4℃
“The Big Easy” EasySep Magnet	18001	STEMCELL Technologies	室温
MOLT-4	CRL-1582	ATCC	液体窒素中
Recombinant Human IL-2	200-02	PEPROTECH	-20℃以下

5-2　細胞数カウント

本手順書は細胞計数装置（自動セルカウンター）NucleoCounter NC-202 を使用する手順を記載しているが、本試験では他メーカーの自動セルカウンターや血球計算盤等の使用も可能である。他メーカーの自動セルカウンターを用いる場合には、メーカー推奨の手順に則った方法で細胞数カウントを行い、当該委託試験終了まで同じ方法で細胞数カウントを行うこととする。

6.　工程：培養 0 日目　T 細胞

6-1　準備

1)　下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
ヒト末梢血 PBMC	CC-2702	Lonza	**指定**
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**
FBS（非働化済のもの）	172012	Sigma®	**指定**
DNase I	2270A	Takara Bio	**指定**
EasySep Buffer	20144	STEMCELL Technologies	**指定**
EasySep Human T cell Isolation Kit	17951	STEMCELL Technologies	**指定**
Dynabeads Human T-Activator CD3/CD28	11131D	Thermo Fisher	**指定**
D-PBS	14249	nacalai	代替可
BSA	A2934	Sigma-Aldrich	**指定**
0.5M EDTA, pH 8.0	AM9260G	Ambion	代替可

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
P1000 フィルターチップ	17014981	RAININ	代替可
P200 フィルターチップ	17014977	RAININ	代替可
50 mL ピペット	768160	Greiner	代替可
25 mL ピペット	170357N	Thermo Fisher	代替可
10 mL ピペット	170356N	Thermo Fisher	代替可
5 mL ピペット	4487	Corning	代替可
薬さじ	－	－	代替可
薬包紙	－	－	代替可
“The Big Easy” EasySep Magnet	18001	STEMCELL Technologies	**指定**
14 mL ポリスチレンチューブ	352057	BD	代替可
75 cm^2 フラスコ	430641U	Corning	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可
50 mL チューブ	91050	Corning	代替可
15 mL チューブ	91015	Corning	代替可
50 mL シリンジ	SS-50ESZ	テルモ	代替可
125 mL 角型培地瓶	2019-0125	Nalgene	代替可
マイレクス-GV, 0.22 µm, PVDF, 33 mm, ガンマ線滅菌済	SLGVR33RS	Millipore	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P1000L マイクロピペット	FA10006P	ギルソン	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
アスピレーター	―	―	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可
天秤	PG603-S	METTLER TOLEDO	代替可

6-2　PBMC の解凍

1) 恒温槽を 37℃に温める。
2) X-VIVO 10 培地 18 mL と FBS 2 mL を 50 mL チューブで混合し、恒温槽で温める。
3) 上記の 2)に DNase I 100 µL を加えてよく混ぜる。以下、「解凍用培地」とする。
4) 液体窒素中に保存している PBMC バイアルを取り出す。蓋が被さった状態で緩めて、バイアル内の圧力を下げ、再び蓋を閉める。蓋の部分を持ち、バイアルを水温 37℃の恒温槽に入れ、小さな氷の塊くらいの大きさになるまで溶かす（ラベルを剥がして状態を目視確認。解凍時間の目安：1〜2 分位）。バイアルをアルコール消毒して安全キャビネット内に入れる頃には完全に溶けているので、1000 µL マイクロピペットを用いて、解凍した細胞懸濁液を新しい 50 mL チューブに入れる。
5) 解凍用培地 1 mL で PBMC バイアルを洗い、上記 4)の 50 mL チューブにゆっくりと移す。
6) 50 mL チューブを軽く振り混ぜながら、もう片方の手で解凍用培地を少しずつ、ゆっくりと 5 mL 加える。
7) さらに解凍用培地を加え、総量 20 mL にする。
8) 室温、420 × g で 5 分間遠心する。
9) 上清を除去し、X-VIVO 10 培地 5 mL を加えて再懸濁する。
10) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液に X-VIVO 10 培地を 100 µL ずつ加えて 2 倍希釈す

る（この希釈は使用するセルカウンターの種類に合わせて実施する。希釈率を変更しても良い）。希釈した細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 4.8 mL × 2 倍希釈*

*X-VIVO 10 培地で 2 倍希釈した場合の式を例示した。実際の希釈率を乗じて算出する。

算出した生細胞数から 5×10^7 cells/mL（6-3 の 1)を参照）になるよう、EasySep Buffer の添加量を計算する。

11) X-VIVO 10 培地 20 mL を加えて、室温、420 × g で 5 分間遠心する。
12) 上清を除去する。

6-3　PBMC から T 細胞分離

1) EasySep Buffer で 5×10^7 cells/mL、液量 0.25～8.5 mL になるように調製する。調製した細胞懸濁液を 14 mL ポリスチレンチューブに入れる。
2) EasySep Buffer の液量 1 mL あたり、Isolation Cocktail 50 µL を加える。よく混合し、室温で 5 分間インキュベーションする。
3) Rapid Spheres を 30 秒間 Vortex する。
4) EasySep Buffer の液量 1 mL あたり、Rapid Spheres 40 µL を加え、よく混合する。
5) 細胞懸濁液が 4 mL 以下のときは総液量 5 mL、細胞懸濁液が 4 mL 以上のときは総液量 10 mL になるよう、下式のように EasySep Buffer の添加量を算出する。算出した EasySep Buffer 量を加え、ゆっくりと 2～3 回ピペッティングして混合する。マグネットの中に 14 mL ポリスチレンチューブを入れて、室温で 3 分間インキュベートする。

EasySep Buffer 添加量 (mL) ＝

総液量（5 mL 又は 10 mL）－EasySep Buffer 量（上記 1)）－

Isolation Cocktail 量（上記 2)）－Rapid Spheres 量（上記 4)）

6) Negative selection：1000 µL マイクロピペット又は 5 mL ピペットを用いて、チューブの中心から細胞懸濁液を回収し、15 mL または 50 mL チューブに移す。
7) さらに、14 mL ポリスチレンチューブをマグネットから外し、EasySep Buffer 総液量（5 mL 又は 10 mL）を加え、チューブの壁面に着いた茶色のビーズを洗いながら、ゆっくりと 2～3 回ピペッティングする。マグネットの中に 14 mL ポリスチレンチュー

ブを入れて、室温で 3 分間インキュベートする。上記 6) の操作を行い、同一の 15 mL または 50 mL チューブに細胞懸濁液を回収する。

8) 新しい 1.5 mL チューブ 1 本に細胞懸濁液を 100 µL サンプリングし、適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）を用いて細胞数カウントを 1 回実施する。ここで、T 細胞数が 2×10^7 cells 以上であることを確認する。2×10^7 cells 未満の場合は、上記 7) の操作を再度繰り返す。これを最後の繰り返しとする。（なお、細胞数カウントはオプションとして行い、カウントしない場合は、7) の操作を必ず行う）
9) 室温、420 × g で 5 分間遠心する。
10) 上清を除去し、X-VIVO 10 培地 5 mL で懸濁する。
11) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液に X-VIVO 10 培地を 100 µL ずつ加えて 2 倍希釈する（この希釈は使用するセルカウンターの種類に合わせて実施する。希釈率を変更しても良い）。希釈した細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 4.8 mL ×2 倍希釈*

*X-VIVO 10 培地で 2 倍希釈した場合の式を例示した。実際の希釈率を乗じて算出する。

回収した T 細胞数が 2×10^7 cells 以上であることを確認する（ただし、2×10^7 cells 未満であっても試験は継続する）。
12) T 細胞懸濁液は使用するまで冷蔵保存する。

6-4 CD3/CD28 ビーズで T 細胞を活性化

1) CD3/CD28 ビーズ洗浄用バッファーの調製

125 mL ボトルに各試薬を下表に従って添加し、フィルター滅菌を行う。ボトルに「ビーズ洗浄用バッファー, 調製日」と記載する。ビーズ洗浄用バッファーは 4℃で保存し、使用期限は 3 ヶ月とする。

試薬名	量
D-PBS	100 mL
BSA	0.1 g
0.5 M EDTA	400 µL

2) CD3/CD28 ビーズを 30 秒以上、Vortex する。
3) 14 mL ポリスチレンチューブに 1000 µL の CD3/CD28 ビーズを移す。
4) ビーズ洗浄用バッファー 5 mL を加えて、5 秒間 Vortex する。
5) マグネットの中に 14 mL ポリスチレンチューブを入れて、1 分間静置する。1000 µL マイクロピペット又は 5 mL ピペットを用いて、チューブの中心からビーズ洗浄用バッファーを除去する。
6) マグネットから 14 mL ポリスチレンチューブを外し、X-VIVO 10 培地 1000 µL で CD3/CD28 ビーズを懸濁する。
7) 75 cm^2 フラスコ 2 個を準備し、下記のように播種する。

	フラスコ 1 個あたり
CD3/CD28 ビーズ	500 µL
T 細胞数	1～2×10^7 cells
液量	20 mL

8) 37℃、5% CO_2 インキュベーターで培養する。

7. 工程：培養 0 日目 スパイク細胞（MOLT-4）

7-1 準備

1) 下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
MOLT-4	CRL-1582	ATCC	**指定**
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**
FBS（非働化済のもの）	172012	Sigma	**指定**

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
75 cm^2 フラスコ	430641U	Corning	代替可
P1000 フィルターチップ	17014981	RAININ	代替可
P200 フィルターチップ	17014977	RAININ	代替可
25 mL ピペット	170357N	Thermo Fisher	代替可
10 mL ピペット	170356N	Thermo Fisher	代替可
5 mL ピペット	4487	Corning	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可
50 mL チューブ	91050	TPP	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P1000L マイクロピペット	FA10006P	ギルソン	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
アスピレーター	―	―	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可

7-2　スパイク細胞（MOLT-4）の解凍とプレ培養

1) 恒温槽を水温 37℃に温める。
2) X-VIVO 10 培地 18 mL と FBS 2mL を 50 mL チューブで混合し、「解凍用培地」とする。
3) 解凍用培地を恒温槽で温める。
4) 新しい 50 mL チューブに、解凍用培地 2 mL を移す。

5) 液体窒素中に保存しているMOLT-4バイアルを取り出す。蓋が被さった状態で緩めて、バイアル内の圧力を下げ、再び蓋を閉める。蓋の部分を持ち、バイアルを水温37℃の恒温槽に入れ、小さな氷の塊くらいの大きさになるまで溶かす（ラベルを剥がして状態を目視確認。解凍時間の目安：1〜2 分位)。バイアルをアルコール消毒して安全キャビネット内に入れる頃には完全に溶けているので、1000 µL マイクロピペットを用いて、解凍した細胞懸濁液を 4)の 50 mL チューブに入れる。

6) 解凍用培地 1 mL で MOLT-4 バイアルを共洗いする。

7) 50 mL チューブを軽く振りながら、もう片方の手で解凍用培地を少しずつ、ゆっくりと加える。

8) 20 mL になるまで解凍用培地を加えたら、室温、420 × g で 5 分間遠心する。

9) 上清を除去し、X-VIVO 10 培地 5 mL を加えて再懸濁する（セルカウンターの種類に合わせて添加培地量を調節する)。

10) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液に X-VIVO 10 培地を 100 µL ずつ加えて 2 倍希釈する（この希釈は使用するセルカウンターの種類に合わせて実施する。希釈率を変更しても良い)。希釈した細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 4.8 mL ×2 倍希釈*

*X-VIVO 10 培地 5 mL で再懸濁し、X-VIVO 10 培地で 2 倍希釈した場合の式を例示した。実際の液量と希釈率を乗じて算出する。

11) X-VIVO 10 培地で 2×10^5 cells/mL になるように調製し、75 cm^2 フラスコに播種する。フラスコあたり、培地量 20 mL 前後を目安にする。

12) 37℃、5% CO_2 インキュベーターで培養する。

8. 工程：培養 3 日目 T 細胞

8-1 準備

1) 下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
6 ウェルプレート	3471	Corning	代替可
“The Big Easy” EasySep Magnet	18001	STEMCELL Technologies	**指定**
14 mL ポリスチレンチューブ	352057	BD	代替可
10 mL ピペット	170356N	Thermo Fisher	代替可
5 mL ピペット	4487	Corning	代替可
P1000 フィルターチップ	17014981	RAININ	代替可
P200 フィルターチップ	17014977	RAININ	代替可
P20 フィルターチップ	17014974	RAININ	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可
50 mL チューブ	91050	TPP	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P1000L マイクロピペット	FA10006P	ギルソン	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
P20L マイクロピペット	FA10003P	ギルソン	代替可
アスピレーター	―	―	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可

8-2　活性化 T 細胞の培養

1) T 細胞が CD3/CD28 ビーズに接着して、凝集塊を形成していることを確認する。
2) マグネットの中に 14 mL ポリスチレンチューブを入れ、ふたを外しておく。
3) CD3/CD28 ビーズの除去：10 mL ピペットで 15～20 回位ピペッティングし、CD3/CD28 ビーズから細胞を剥がしてシングルセルにする。細胞の状態により細胞とビーズの分離が難しい場合には、5 mL ピペットを用いて、再度 15～20 回位ピペッティングする。上記 2)のチューブに細胞懸濁液 10 mL を入れ、1 分程度静置する。5 mL ピペットを用いて、チューブの中心から細胞懸濁液を回収し、50 mL チューブに移す。CD3/CD28 ビーズは可能な範囲で除去する。
4) 上記 3)を繰り返す。すべての細胞懸濁液を回収、及び CD3/CD28 ビーズを除去し終えたら、50 mL チューブに X-VIVO 10 培地を添加して 40 mL までメスアップする。
5) 室温、420 × g で 5 分間遠心する。
6) 上清を除去し、X-VIVO 10 培地 10 mL に懸濁する。
7) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液に X-VIVO 10 培地を 100 µL ずつ加えて 2 倍希釈する（この希釈は使用するセルカウンターの種類に合わせて実施する。希釈率を変更しても良い）。希釈した細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 9.8 mL ×2 倍希釈*

*X-VIVO 10 培地で 2 倍希釈した場合の式を例示した。実際の希釈率を乗じて算出する。

8) 上記 7) から 2.0 ×10^7 cells 分の細胞懸濁液を取り 50 mL チューブに移し、総液量 40 mL になるよう X-VIVO 10 培地を加えて、5×10^5 cells/mL になるように調製する。

9) 6 ウェルプレートを 3 枚準備する。

10) 6 ウェルプレートの 1 ウェルあたり、下記のように播種する。これを 18 ウェル分行う。

	1 ウェルあたり
T 細胞数	1×10^6 cells
液量	2 mL

11) 37℃、5% CO_2 インキュベーターで培養する。

9. 工程：培養 3 日目 スパイク

9-1 準備

1) 下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**
Recombinant Human IL-2	200-02	PEPROTECH	**指定**

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
5 mL ピペット	4487	Corning	代替可
P1000 フィルターチップ	17014981	RAININ	代替可
P200 フィルターチップ	17014977	RAININ	代替可
P20 フィルターチップ	17014974	RAININ	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可
50 mL チューブ	91050	TPP	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P1000L マイクロピペット	FA10006P	ギルソン	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
P20L マイクロピペット	FA10003P	ギルソン	代替可
アスピレーター	―	―	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可

9-2　T 細胞に MOLT-4 をスパイク

1) rIL-2 の調製：10 µg の rIL-2 を X-VIVO 10 培地 1 mL で溶解し、10 µg/mL rIL-2 溶液を調製する。100 µL ずつ分注し、-20˚C 以下で保存する。分注した rIL-2 溶液の使用期限は 3 ヶ月とする。
2) プレ培養した MOLT-4 を 50 mL チューブに回収し、室温、420 × g で 5 分間遠心する。
3) 上清を除去し、X-VIVO 10 培地 5 mL に懸濁する。

4) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液に X-VIVO 10 培地を 900 µL ずつ加えて 10 倍希釈する（この希釈は使用するセルカウンターの種類に合わせて実施する。希釈率を変更しても良い）。希釈した細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 4.8 mL ×10 倍希釈*

*X-VIVO 10 培地で 10 倍希釈した場合の式を例示した。実際の希釈率を乗じて算出する。

5) スパイクあり：X-VIVO 10 培地を用いて、最初に 5×10^5 cells/mL の MOLT-4 細胞懸濁液を 1000 µL 調製し、100 µL の細胞懸濁液を 900 µL の培地で段階希釈する。8-2 の工程で 6 ウェルプレートに播種した T 細胞に MOLT-4 を下表のように播種する。

スパイク細胞数	1×10^4 cells	1×10^3 cells	1×10^2 cells	1×10^1 cells
MOLT-4 濃度	5×10^5 cells/mL	5×10^4 cells/mL	5×10^3 cells/mL	5×10^2 cells/mL
1 ウェルあたりの添加量	20 µL	20 µL	20 µL	20 µL

6) スパイクなし：MOLT-4 をスパイクしない系は、rIL-2 添加なし（以下、(-)と表記する。）と rIL-2 添加あり（以下、(+)と表記する。）がある。rIL-2(-)の系はそのまま培養を継続させ、rIL-2(+)の系は、1 ウェルあたり 10 µg/mL rIL-2 溶液 6 µL を添加する。

7) 37℃、5% CO_2 インキュベーターで培養する。

10. 工程：培養 5 日目 拡大培養

10-1 準備

1) 下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**
Recombinant Human IL-2 （10 μg/mL に調製したもの）	200-02	PEPROTECH	**指定**

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
6 ウェルプレート	3471	Corning	代替可
2 mL ピペット	356507	Falcon	代替可
5 mL ピペット	4487	Corning	代替可
P200 フィルターチップ	17014977	RAININ	代替可
P20 フィルターチップ	17014974	RAININ	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
P20L マイクロピペット	FA10003P	ギルソン	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可

10-2 拡大培養

1) 6 ウェルプレートで培養している細胞懸濁液を P1000 ピペットでよく混合する。

2) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10～20 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、本試験の rIL-2(-)の系では、正常 T 細胞の生存率が経時的に減少し、生細胞濃度も低下するため、血球計算盤を使用する場合は、全区画でのカウントを行う。
3) 新しい 6 ウェルプレートを 3 枚準備する。
4) 新しい X-VIVO 10 培地を 1 ウェル当たり 1.5 mL を添加する。rIL-2(+)の系は 10 µg/mL rIL-2　溶液 4.5 µL を添加する。
5) 2 mL ピペットで細胞懸濁液をよく混合し､1 ウェル分の細胞懸濁液 1.5 mL を加えて、2 倍希釈とする。
6) 37℃、5% CO_2 インキュベーターで培養する。

11. 工程：培養 7 日目

11-1 準備

1) 下表に従って、原料を準備する。

品名	型番	メーカー	指定/代替可
X-VIVO 10 培地 (Serum-Free Medium With Gentamicin and Phenol Red)	04-380Q	Lonza	**指定**
Recombinant Human IL-2 （10 µg/mL に調製したもの）	200-02	PEPROTECH	**指定**

2) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
75 cm^2 フラスコ	430641U	Corning	代替可
2 mL ピペット	356507	Falcon	代替可
25 mL ピペット	170357N	Thermo Fisher	代替可
P1000 フィルターチップ	17014981	RAININ	代替可
P200 フィルターチップ	17014977	RAININ	代替可
P20 フィルターチップ	17014974	RAININ	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可

3) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P1000L マイクロピペット	FA10006P	ギルソン	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
P20L マイクロピペット	FA10003P	ギルソン	代替可
37℃ 恒温槽	SDN-B	タイテック	代替可
NucleoCounter	NC-202	Chemometec	代替可

11-2 拡大培養

1) 6 ウェルプレートで培養している細胞懸濁液を、P1000 ピペットでよく混合する。
2) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等では 10～20 µL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、本試験の rIL-2(-)の系では、正常 T 細胞の生存

率が経時的に減少し、生細胞濃度も低下するため、血球計算盤を使用する場合は、全区画でのカウントを行う。

3) 75 cm^2 フラスコを 18 個準備する。
4) 75 cm^2 フラスコ 1 個あたり、新しい X-VIVO 10 培地 18 mL を添加する。rIL-2(+)の系は 10 µg/mL rIL-2 溶液 54 µL を添加する。
5) 18 mL の培地が入った 75 cm^2 フラスコに、1 ウェル分の細胞懸濁液 2 mL を加えて、10 倍希釈とする。
6) 37℃、5% CO_2 インキュベーターで培養する。

12. 工程：10、14、17、21、24、28 日目

12-1 準備

1) 下表に従って、資材を準備する。

品名	型番	メーカー	指定/代替可
10 mL ピペット	170356N	Thermo Fisher	代替可
P200 フィルターチップ	17014977	RAININ	代替可
細胞数カウント用カセット Via2-Cassette	941-0024	Chemometec	代替可
1.5 mL チューブ	0030 125.150	エッペンドルフ	代替可

2) 下表に従って、機器類を準備する。

品名	型番	メーカー	指定/代替可
安全キャビネット	CLASSIITYPEA2 MHE-132AJ-PJ	日立 パナソニック	代替可
電動ピペッター	XP 型	Drummond	代替可
P200L マイクロピペット	FA10005P	ギルソン	代替可
NucleoCounter	NC-202	Chemometec	代替可

12-2 細胞数カウント

1) 75 cm^2 フラスコで培養している細胞懸濁液を 10 mL ピペットでよく混合する。
2) 新しい 1.5 mL チューブ 2 本に細胞懸濁液を 100 µL ずつ（計 200 µL）サンプリングする。サンプリングした細胞懸濁液を適量（例えば NC-202 では 100 µL、Countess 等で

は 10～20 μL）用いて細胞数カウントを実施する。細胞数カウントは 1.5 mL チューブ 1 本につき 1 回実施し、合計 2 回実施する。2 回のカウントから平均値（総細胞数、生細胞数、生存率）を求める。なお、本試験の rIL-2(-)の系では、正常 T 細胞の生存率が経時的に減少し、生細胞濃度も低下するため、血球計算盤を使用する場合は、全区画でのカウントを行う。なお、総細胞数、生細胞数は、次の式で求める。

細胞数 (cells) ＝ 細胞濃度 (cells/mL) × 液量 20 mL*

*各日（10、14、17、21、24、28 日目）の液量は 20 mL とする。

3) 37℃、5% CO_2 インキュベーターで培養する。以降、培地交換は行わず、細胞数カウントを経時的に実施する。

13. 記録書など

13-1 本試験を実施する委託試験実施機関は、国立医薬品食品衛生研究所との間で予め取り決めた方法にて、記録を行う。

13-2 本試験を実施した委託試験実施機関は、試験終了した後、当該試験結果を速やかに国立医薬品食品衛生研究所へ提出するものとする。

14. 記録の保管

14-1 本試験に関する全ての生データ、記録書（実験ノート、電子媒体記録を含む。）等は、本試験を実施する委託試験実施機関で定める手順に基づき、保管及び管理を行う。

以上

学会等発表実績

1. 学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
Japan’s Recent Progress in Induced Pluripotent Stem Cell (iPSC)-based Therapies.（口頭）	Sato Y.	The 2022 World Stem Cell Summit	2022.6.11	国外
Regulatory Science for Ensuring the Quality and Safety of Cell Therapy Products and its Interface with Basic Stem Cell Science.（口頭）	Sato Y.	International Society for Stem Cell Research 2022	2022.6.15	国外
Safety of cell therapy products: *In-vitro* methods to assess the tumorigenicity of human cell-based therapeutic products.（ポスター）	Henry M, Lemmens M, Sato Y, Marginean D, Harada K, Watanabe T, Bando K, Terai O, Moss D, Chen C, Nicholas N, Mouriès LP, Smart M, Libertini S, Yasuda S.	International Society for Stem Cell Research 2022 Annual Meeting	2022.6.17	国外
Regulatory Science Research for Clinical Applications of Products Derived from Human Induced Pluripotent Stem Cells.（口頭）	Sato Y.	KSSCR 2022 Annual Meeting	2022.8.12	国外
ICH Q5A(R2) 「ヒト又は動物細胞株を用いて製造されるバイオテクノロジー応用医薬品のウイルス安全性評価」のステップ 2 公開ドラフトの概要について.（口頭）	佐藤陽治	日本 PDA 製薬学会第 29 回年会	2022.11.29	国内
間葉系幹細胞の薬理効果に寄与するバイオマーカーの効率的な同定法の開発（ポスター）	三浦巧, 河野掌, 高野慈美, 黒田拓也, 山本由美子, 草川森士, 森岡勝樹, 菅原亨, 平井孝昌, 安田智, 澤田留美, 松山さと子, 川路英哉, 粕川雄也, 伊藤昌可, Jay W Shin, 梅澤明弘, 河合純, 佐藤陽治	日本再生医療学会第 2 回科学シンポジウム	2022.12.2	国内

細胞加工製品の品質評価におけるサンプルサイズの設定について（ポスター）	草川森士，安田智，佐藤陽治	日本再生医療学会第 2 回科学シンポジウム	2022.12.2	国内
神経細胞製造の原料としてのヒト多能性幹細胞の品質試験法の開発（ポスター）	黒田拓也，安田智，松山さと子，三浦巧，澤田留美，松山晃文，森岡勝樹，粕川雄也，山本由美子，川路 英哉，伊藤昌可，阿久津英憲，河合純，佐藤陽治	日本再生医療学会第 2 回科学シンポジウム	2022.12.2	国内
単一細胞遺伝子発現解析による間葉系幹細胞の血管新生能予測バイオマーカーの探索（口頭）	三浦巧，河野掌，高野慈美，黒田拓也，山本由美子，草川森士，森岡勝樹，菅原亨，平井孝昌，安田智，澤田留美，松山さと子，川路英哉，粕川雄也，伊藤昌可，Jay W Shin，梅澤明弘，河合純，佐藤陽治	第 22 回日本再生医療学会総会	2023.3.23	国内
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細胞加工製品の品質評価におけるサンプルサイズの設定について―形質転換細胞検出試験を例に―（ポスター）	草川森士，安田智，佐藤陽治	第 22 回日本再生医療学会総会	2023.3.25	国内

iPS 細胞に感染できる C 型肝炎ウイルス亜株の分離と性状解析	水上智晴，白砂圭崇，深澤秀輔，長瀬翔太朗，清水芳実，脇田隆字，鈴木哲朗，谷英樹，近藤昌夫，黒田拓也，安田智，佐藤陽治，花田賢太郎，深澤征義	日本薬学会第 143 年会	2023.3.28	国内

2. 学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells.	Hirai T, Yasuda S, Umezawa A, Sato Y.	Stem Cell Rep	2023年	国外
Evaluation of the reproducibility and positive controls of cellular immortality test for the detection of immortalized cellular impurities in human cell-processed therapeutic products.	Hirai T, Kono K, Kusakawa S, Yasuda S, Sawada R, Morishita A, Hata S, Wakita A, Kageyama T, Takahashi R, Watanabe S, Shiraishi N, Sato Y.	Regen Ther	2022年	国内
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International evaluation study of a highly efficient culture assay for detection of residual human pluripotent stem cells in cell therapies.	Watanabe T, Yasuda S, Chen CL, Delsing L, Fellows MD, Foldes G, Kusakawa S, Mouries LP, Sato Y.	Regen Med	2022年	国外
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再生医療におけるわが国の法令および規制	髙田のぞみ，佐藤陽治	日本医師会雑誌	2022年	国内
ヒト細胞加工製品の製造における *in vitro* 細胞特性評価の重要性	佐藤陽治	PHARM STAGE	2022年	国内
遺伝子改変されたブタ心臓のヒトへの移植について	平井孝昌，佐藤陽治	医薬品医療機器レギュラトリーサイエンス	2022年	国内
細胞加工製品の腫瘍形成リスクの合理的評価を目指して(1)	田中直子，阿部浩幸，荒木紀帆，石井匡，上野高嗣，黒田拓也，佐治大介，中村和靖，南保泰希，西田仁，坂東清子，藤田大樹，松浦哲也，三浦巧，三木健次，望月秀美，安田智，吉本将成，渡辺夏巳，佐藤陽治	再生医療	2023年	国内
薬剤学で切り拓く創薬モダリティの未来（3）細胞医薬・細胞製剤の現状と薬剤学で切り拓く未来	樋口ゆり子，草森浩輔，佐藤陽治，坂東博人	薬剤学	2023年	国内

Country-specific regulation and international standardization of cell-based therapeutic products derived from pluripotent stem cells

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Summary

Currently, many types of cell-based therapeutic products (CTPs) derived from pluripotent stem cells (PSCs) are being developed in a lot of countries, some of which are in clinical trial stages. CTPs are classified differently in different countries and regions. The evaluation of their efficacy, safety, and quality also differs from that for conventional small-molecule drugs and biopharmaceuticals, which reflects the complex properties of living cells and unmet medical needs. Since there are no international guidelines to evaluate CTPs, including PSC-derived products, it is necessary to be aware of differences in relevant laws and regulations in different countries and regions. International consortia are organized and actively working to standardize/harmonize the evaluation methods and regulations to facilitate the development and global distribution of PSC-derived CTPs. In this paper, we outline the regulations related to PSC-derived CTPs in the ICH founding regions (US, EU/UK, Japan) and introduce representative consortia working on their standardization.

Introduction

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are types of pluripotent stem cells (PSCs) capable of infinite self-renewal and differentiation into different cell types. By exploiting the characteristics of PSCs, target cells can be differentiated from human ESCs/iPSCs and used as therapeutic products for intractable diseases in regenerative medicine and cell therapy. Cell-based therapeutic products (CTPs), including PSC-derived products, are different from small-molecule and antibody drugs and are also commonly regarded as innovative pharmaceuticals. As CTPs are composed of living cells and vary widely in raw materials, manufacturing processes, final product form, and disease treatment, a case-by-case approach should be applied to each product to ensure its quality and safety. Therefore, conventional test methods and good practices applied to pharmaceuticals are often not applicable to CTPs. Furthermore, there is no international consensus on the evaluation and good practices related to the efficacy, safety, and quality of CTPs and no internationally available regulatory guidelines have been issued.

In the United States (US), the European Union (EU) (and the United Kingdom (UK)), and Japan, the three founding members of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), regulations are established according to the product categories defined in each country and region. CTPs are usually classified according to whether they contain manipulated/processed cells, or whether they are intended for homologous use. In the US, therapeutic products containing cells with more-than-minimal manipulation, including PSC-derived products, are deemed human cells, tissues, and cellular and tissue-based products under Section 351 of The Public Health Service Act (351 HCT/Ps) and classified as biological products or medical devices, whereas CTPs containing substantially manipulated/processed cells are classified as advanced therapy medicinal products (ATMPs) and regenerative medical products in the EU (and the UK) and Japan, respectively. CTPs are approved for marketing after undergoing a review process that is unique to each country or region. Also, clinical trials using CTP involving transplantation or surgical procedures must comply with national regulations regarding ethics. In addition, the rare and serious disease areas that are often the targets of CTPs often do not have sufficient patient populations for their clinical development. For these reasons, time is required to collect and evaluate data for efficacy validation and thus, each country or region has its own system for the early approval of CTPs (Sato *et al.*, 2019; Yoneda *et al.*, 2021). In this current situation, standardization of manufacturing and evaluation

techniques and harmonization/convergence of regulations are effective approaches to accelerate the product development and expedite approval. Discussions are currently being held on a wide variety of topics related to regenerative medicine, including definitions of terms, cell sampling and storage methods, manufacturing processes, and evaluation tests by various international consortia that collaborate with each other.

This paper describes the following: 1) the review systems, regulations, and guidelines related to the approval process for CTPs, including PSC-derived products, in the US, EU, and Japan; 2) expedited approval systems in each country and region that were developed in consideration of the characteristics of CTPs and unmet medical needs; and 3) representative international platforms working on the technical standardization or regulatory harmonization/convergence of CTPs.

The principle of regulation: a risk-based approach

The risk-based approach is a general principle for the regulation of pharmaceutical products in the three ICH founding states and has also been adopted in the ICH Guidance on Quality Risk Management (Q9). The risk-based approach establishes regulatory policies and content by scientifically evaluating the degree of impact of a product, based on the identification of risk factors inherent to the property of each product with respect to efficacy, safety, and quality (Table 1. #1, #13, #17–19) (FDA/CBER, 1997; EMA/CAT/CPWP, 2013; MHLW/PFSB, 2012a-c; Hayakawa *et al.*, 2015a-c). It is difficult to achieve a uniform quality of CTPs, including PSC-derived products, owing to various factors such as differences in cell donors, as well as complexity and activity of cells as living organisms. In addition, raw materials, manufacturing processes, final product forms, and clinical use vary widely from product to product. Therefore, the quality profiles and uses of final products are highly diverse, and the associated risk factors and risk severity vary among the products. To obtain a risk profile, it is necessary to scientifically evaluate the risk factors for each product from an early stage of development.

Regional regulations of PSC-derived CTPs

The United States

Product classification in the US

Cells, tissues, and cell/tissue processed products are called 361 HCT/Ps if they meet all the requirements

presented in Section 361 of the of the Public Health Service Act, such as that the cells are not subjected to more than minimal manipulation and that the product is intended for homologous use only. When none of these are the case, the products are referred to as 351 HCT/Ps. Minimal manipulation means processing that does not alter the relevant biological characteristics of the cells or tissues. PSC-derived CTPs are therefore considered to be 351 HCT/Ps as their manufacturing process is not regarded to involve "minimal manipulation" but "more than minimal manipulation" (Table 1. #1 and #7) (FDA/CBER, 1997; FDA/CBER/CDRH/OCP, 2020; Federal Food, Drug, and Cosmetic Act(United States code, Title 21), 1938). Based on the primary mode of action principle, 351 HCT/Ps are classified as either biologics (pharmacological, immunological, and/or metabolic effects) or medical devices (structural and/or physical effects). In the case of CTPs, which are difficult to classify as biologics or medical devices, the FDA's Office of Combination Products (OCP) determines the classification.

Clinical trials

Any clinical trials for 351 HCT/Ps must be conducted in compliance with the ICH Good Clinical Practice (GCP) guidelines. In addition, following the applications, biological product: Investigational New Drug (IND) and medical device: Investigational Device Exemption (IDE) from the Food and Drug Administration (FDA), approval is needed to conduct clinical trials for 351 HCT/Ps. For the practical application of 351 HCT/Ps, developers are entitled to receive support, for example, through a program that allows them to consult the FDA.

Expedited marketing authorization system in the US

It is necessary to apply for a Biologics License Application (BLA) or a Premarket Approval (PMA) after clinical trials to obtain marketing authorization for HCT/Ps. The FDA has established the Office of Cell, Tissue and Gene Therapy (OCTGC) of the Center for Biologics Evaluation and Research (CBER) as the point of contact for reviewing 351 HCTP/s, regardless of whether they are a biologic or a medical device.

The 21st Century Cures Act was established in 2016 with the primary objective of addressing unmet medical needs and incorporating the patient's perspective into the regulation of drugs and medical devices, building 21st century medical care, promoting rational clinical trials, supporting continued innovation by government agencies, and reforming the regulatory process. The content related to regenerative medicine is

described in Section 3033–3036 of the Act. As described in Section 3033 of the "Accelerated Approval for Regenerative Medicine Advanced Therapies", the Regenerative Medicine Advanced Therapies (RMAT) designation, which enables accelerated approval of HCT/Ps, including PSC-derived products, was stipulated. The requirements for RMAT are as follows: 1) the product is intended to be a regenerative therapy; 2) the product is intended to treat, correct, repair, or cure a serious or life-threatening disease or condition; or 3) preliminary clinical results indicate that the product has the potential to address an unmet medical need for such diseases or conditions. Priority review or accelerated approval may be applied if the RMAT designation is granted after submitting an IND application. Priority review is a program that reduces the review period from the usual 10 months to approximately 6 months (Food and Drug Administration Modernization Act of 1997, 1997). In addition, accelerated approval is a program under which marketing authorization of a drug may be granted for a serious or fatal disease if a well-controlled clinical trial demonstrates efficacy for a surrogate endpoint or for an endpoint other than survival or irreversible conditions (Code of Federal Regulation under Title 21, 21 CFR). Products designated as RMAT are eligible for consultations regarding the surrogate/intermediate endpoint for Accelerated Approval. Further clinical trials are required to confirm the efficacy of the product. Approval for the product is revoked if the FDA determines that the efficacy of the product is inadequate, based on the results of post-marketing surveillance reports. In contrast, if the FDA determines that the product is effective, the obligation for post-marketing surveillance ends.

HCT/Ps classified as medical devices are eligible for humanitarian device exemption (HDE) under the humanitarian use device (HUD) designation program. An HUD is defined as a medical device that is beneficial to the patient in the treatment or diagnosis of a disease or condition that affects or develops in 4,000 or fewer people per year in the US, for which no other effective devices are available. An HUD is designated by the Office of Orphan Product Development of the FDA. Once an HDE is granted to an HUD-designated medical device, efficacy data may be substituted to explain its probable benefit during the regulatory review process. Therefore, it is not necessary to submit the results of clinical studies that scientifically demonstrate the efficacy of the device. However, it should be noted that medical devices approved under an HDE must be approved by the Institutional Review Board (IRB) prior to use at medical institutions (Code of Federal Regulation under Title 21).

Guidelines for evaluating efficacy, safety, and quality

In the US, although no specific guidance for PSC-derived CTPs with clinical application has been issued by regulatory bodies, several guidelines for HCT/Ps are available. ESCs and iPSCs can be the raw materials for allogeneic PSC-derived CTPs. As virus clearance during the manufacturing process of CTPs is impossible, the eligibility of the donor is very important particularly in the case of allogeneic products. In the "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" (FDA/CBER, 2007) (Table 1. #2), information on donor eligibility testing and communicable disease agents are described in 21 CFR Part 1271.3(r) (Code of Federal Regulation under Title 21). As with general pharmaceuticals, the efficacy, safety, and quality of CTPs is verified during preclinical studies; however, the intrinsic material composition and putative MOAs differ from those of small-molecule drugs, biopharmaceuticals, and medical devices. Therefore, the traditional approach using in preclinical toxicity testing is often inappropriate for evaluating the safety of CTPs. With CTPs, it is necessary to consider the impacts of biologically active substances secreted from cells and contamination with tumorigenic cells, including transformed cells and undifferentiated cells. In addition, if there is a possibility that non-cellular components and/or impurities originating during the manufacturing process are present in CTPs, their toxicity should be evaluated. The Preclinical Assessment of Investigational Cellular and Gene Therapy Products is issued by the CBER and OCTGT, and it provides information about the design of preclinical studies for HCT/Ps (e.g., animal species selection, proof-of-concept studies, and toxicology studies) (Table 1. #5) (FDA/CBER, 2013). The evaluation of CTPs is influenced by product origin, such as donor and tissue sources, level of manipulation, stage of differentiation at the time of administration, cellular heterogeneity, and batch variability. In addition, the characteristics of CTPs, such as engraftment at the site of administration or migration to other sites, can be affected by the surrounding microenvironment because they have unique complexities because of the dynamic nature of living cells. This also affects teratoma/tumor formation due to the residual undifferentiated PSCs and a range of different transformed cell types arising as a result of genetic variations, which is a concern for PSC-derived products. Therefore, the quality and efficacy can affect the fate of the product after administration, including its distribution, differentiation, integration, and tumorigenicity, and should be verified in preclinical studies. "The Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products" (FDA/CBER, 2015) (Table 1. #6) states that an early phase clinical trial design should include

dosimetry, a feasibility assessment, and an activity assessment. The objectives of early phase trials are to assess the risks of the trial and protect the subjects. In particular, the priority of first in-human trials in to evaluate safety, which includes an assessment of the nature and frequency of potential adverse effects and an estimation of their relationship to the dosage (Code of Federal Regulation under Title 21). Potency assays are required for biological products and CGT applications are also described in the document "Potency Tests for Cellular and Gene Therapy Products" (FDA/CBER, 2011) (Table 1. #3). However, the manufacturing process of HCT/Ps has a significant impact on efficacy, safety, and quality. The manufacture of 351 HCT/Ps as biologics and medical devices requires compliance with current Good Manufacturing Practice (cGMP) and the current quality system regulation (QSR), respectively, as well as the current Good Tissue Practice (cGTP) (Code of Federal Regulation under Title 21, 21 CFR). These documents provide good practices for standard operating procedures (SOPs), process validation, and instrument calibration/validation to maintain a sterile manufacturing environment. A unique aspect of the cGTP is that it is based on preventing the spread of infectious diseases, as the products are human cells and tissues. To help better understand the relevant guidelines, a Q&A style guidance document has been published (Table 1. #8) (FDA/CBER, 2022a).

Recommendations to the FDA by the Cellular, Tissue, and Gene Therapies Advisory Committee

The Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) is an advisory committee of the FDA, and its expert members review and evaluate data on the efficacy, safety, and appropriate use of human cells, tissues, and other materials for regenerative medicine. The results of their discussions are then used as recommendations for the FDA. Cellular therapies derived from human ESCs were discussed at the CTGTAC Meeting #45 in 2008, and they then mainly summarized the study designs that avoid adverse events that are mainly attributable to the teratoma-forming potential of ESCs (Table 1. #4) (FDA Cellular, Tissue, and Gene Therapies Advisory Committee, 2008). To manufacture human ESC-derived CTPs, it is important to minimize the number of undifferentiated human ESCs. Tests used to detect unacceptable amounts of undifferentiated human ESCs and other cellular impurities are required to evaluate the quality of human ESC-derived CTPs. In addition, the selection of animal species and models, cell dose, and site of administration (niche) should be considered during preclinical studies to assess the potential for tumorigenesis and inappropriate differentiation at nontarget sites. These factors significantly affect the efficacy and safety of the

192 product, as they are involved in cell engraftment, differentiation, and migration. These requirements also apply
193 to clinical studies. Clinical trials using human ESC-derived CTPs should be conducted in patients with the target
194 disease. In clinical trials, non-invasive techniques, such as imaging and blood markers should be used to detect
195 potential adverse events, including the formation of teratomas and other tumors. However, key parameters for
196 clinical trials must be carefully chosen because early signs of adverse events may be undetectable. Appropriate
197 study designs should be established based on scientific evidence.
198 Although the opinion of the CTGTAC with respect to human ESC-derived CTPs could be applicable to
199 iPSC-derived CTPs, as far as we know, no CTGTAC meeting discussions have focused on iPSC-derived CTPs.
200 Different from ESC, iPSC can be generated by the reprogramming of somatic cells and clinically used for
201 autologous transplantation. Additional discussions about the clinical application of iPSC-derived products are
202 expected.
203
204 *Standardization of relevant technologies in the US*
205 Section 3036 of the 21st Century Cures Act "Standards for Regenerative Medicine and Regenerative
206 Advanced Therapies," states that efforts shall be made to formulate the definition of terminology and standards
207 to support the development, evaluation, and review of regenerative medicine therapies/advanced regenerative
208 therapies, including product manufacturing processes and controls. This formulation is based on regulations
209 made through public processes with the National Institute of Standards and Technology (NIST). The Standards
210 Coordinating Body (SCB) is a new consortium established under the leadership of the Science and Technology
211 Committee for the Alliance for Regenerative Medicine (ARM). The SCB, in partnership with the NIST and
212 FDA, contributes to international standardization activities in cooperation with industry associations. For
213 example, the members of SCB, as well as groups such as FDA and standards committee members from various
214 countries, join in the International Organization for Standardization (ISO) Technical Committee (TC) 276,
215 which is discussed below.
216 The policy states that the preferential usage of standards, which are internationally harmonized and
217 consistent with US law, increases the availability of reviews and leads to a shortened time-to-market period. For
218 the purpose of facilitating the development and assessment of regenerative medicine therapy products, the
219 FDA's CBER has recently issued a draft guidance document on the use of voluntary consensus standards for

regenerative medicine therapies (Table 1. #9) (FDA/CBER, 2022b). This guideline describes the requirements that need to be met in the process of developing voluntary consensus standards, with respect to how standards are recognized and how they will be reviewed for recognition.

The European Union

Product classification

In the EU, CTPs, including PSC-derived products, are handled as ATMPs, which require regulatory approval (The European Parliament and the Council of the European Union, 2007). ATMPs are human medicines based on genes, tissues, or cells that offer groundbreaking new opportunities for the treatment of diseases and injuries. ATMPs can be classified into three main types: gene therapy medicines, somatic-cell therapy medicines, and tissue-engineered medicines. The classification of somatic-cell therapy medicines and tissue-engineered medicines is based on the principle of the primary mode of action. Namely, the expected functions of somatic-cell therapy medicines and tissue-engineered medicines are "pharmacological, immunological, or metabolic" and "structural or physical", respectively (Table 1. #12) (European Medicines Agency (a); EMA/CAT, 2011). In scientific recommendations of EMA, retinal pigment epithelial cells derived from iPSCs were classified as tissue-engineered products (European Medicines Agency, 2014). For the handling of ATMPs in the United Kingdom (UK), which has left the EU, please refer to the guidance which is described by Cell and Gene Therapy Catapult (Cell and Gene Therapy Catapult, 2021).

Clinical trials in the EU

The Clinical Trials Regulation (The European Parliament and the Council of the European Union, 2014) was established in place of the Clinical Trials Directive (The European Parliament and the Council of the European Union, 2001a) to protect the right, safety, dignity, and well-being of subjects in clinical trials, and it applies to all clinical trials that involve the use of medicinal products for humans in the EU. The aim of the Clinical Trials Regulation is to harmonize the processes for assessing and supervising clinical trials. To date, the evaluation, authorization, and supervision of clinical trials are the responsibilities of EU Member States and European Economic Area countries (The European Parliament and the Council of the European Union, 2001a). Manufacturers of medicinal products for human use had to submit clinical trial applications separately

to the national competent authorities and ethics committees in each country to run a clinical trial. Therefore, even if the same clinical trial applications are submitted to different member countries, the conclusions may differ from country to country. To obtain approval to conduct a clinical trial in up to 30 EU/EEA countries, the Clinical Trials Regulation, which entered into application on 31 January 2022, allows manufacturers to simultaneously submit online applications using the same documentation via the Clinical Trials Information System (CTIS) (European Medicines Agency (b)). When applying for multinational trials, all concerned Member States of the EU share any considerations and coordinately review the application of Part I, while each Member States assesses the application of Part II with respect to its own territory (The European Parliament and the Council of the European Union, 2014). The reporting Member State and each Member States concerned then notify the manufacturers of their conclusion via CTIS. ATMPs, including PSC-derived products, are reviewed for regulatory approval by the European Medicines Agency (EMA); however, the EMA has no authority over clinical trials. The EMA is responsible for maintaining CTIS, and the European Commission (EC) oversees the implementation of the Clinical Trials Regulation (European Medicines Agency, 2022).

As in the US, clinical trials/research must be conducted in accordance with ICH GCP guidelines, regardless of whether they are non-commercial or commercial. Considerations relating to clinical trial designs and the quality of investigational ATMPs (iATMP) are stated in the GCP for ATMPs developed in the EU (Table 1. #16) (European Commission, 2019). When conducting clinical studies on ATMPs, an Investigational Medicinal Product Dossier (IMPD) should be prepared. The IMPD is a summary of the investigational drug and describes the drug substance (DS), drug product (DP), device, etc., using the European Directorate for the Quality of Medicines & Health Care (EDQM) standard terms. The DS is defined as the processed starting material used in manufacturing and is associated with information on the name, the type of modality, and the mode of action. The DS provides most of the information on ATMPs. The DP provides information about the route of administration, the sterility, and the dilution of the ATMPs. For a particular investigational ATMP (iATMP), the starting material, active substance, and final product may be closely related or nearly identical. If possible, active, intermediate, and final products should be identified. The IMPD should also describe the process validation performed and summarize the key points relevant to the understanding of the selected product development approach (Centre for Advanced Medical Products, 2019).

Exception systems for the use of ATMPs

For ATMPs to be marketed in the EU, their efficacy, safety, and quality need to be scientifically evaluated for approval review, which is performed by the EMA under the delegation of the EC. The Committee for Medicinal Products for Human Use (CHMP) is responsible for the regulatory review of drugs and medical devices within the EMA; however, as the evaluation of ATMPs is more specialized and involves multidisciplinary perspectives compared to that for conventional drugs and medical devices, the Committee for Advanced Therapies (CAT) was organized as an advisory body for the CHMP. An approval review is conducted by the CHMP based on the evaluation of efficacy, safety, and quality from the CAT. The EC then makes an approval decision based on an evaluation letter prepared by the CHMP. To resolve these requirements, evaluate ATMPs within the EU, and prompt their marketing, the efficacy, safety, and quality of ATMPs are evaluated directly by the CAT. Even ATMPs that are to be distributed in only one member country must be evaluated by the CAT and reviewed by the CHMP.

In the EU, the PRIME program supports the development of drugs for unmet medical needs. PRIME is designed to enhance networking and promote early meetings with developers of promising drugs, with the aim of strengthening support for the development of drugs to treat currently unmet medical needs. This program is expected to optimize the development plan, accelerate the evaluation of new drugs, and make them more readily available to patients. The eligibility of ATMPs for PRIME is determined according to the extent to which they can be applied to unmet medical needs, their expected efficacy, and their improvement over existing treatments and methods. In addition to the data on clinical activity, a summary of all available safety data obtained in non-clinical and clinical settings should also be provided (Table 1. #14) (EMA/CHMP, 2015). ATMPs that qualify for PRIME may be eligible for accelerated assessment with a shorter review period at the time of regulatory approval (European Medicines Agency (c)). In addition, ATMPs are exempt from review by the EMA if there are no other approved drugs for particular conditions with high unmet medical needs, and the product is produced non-repetitively at a single hospital within the same member country for a specific patient population (The European Parliament and the Council of the European Union, 2007). This type of approval is called "Hospital Exemption"; however, even products eligible for the Hospital Exemption must be reviewed and approved for manufacturing, quality, and pharmacovigilance by the agency in the country of production and distribution and be produced in good manufacturing practice (GMP)-authorized sites. A recommendations and

position paper for the use of Hospital Exemptions is issued from ARM (which involves 350+ members worldwide and 70+ members across 15 European countries) and the European Association for Bioindustries, respectively (Alliance for Regenerative Medicine, 2020; European Association for Bioindustries, 2020). The "Specials" Exemption is another system of exceptional use for specific patients (The European Parliament and the Council of the European Union, 2001b; Medicines and Healthcare products Regulatory Agency, 2014). The Hospital Exemption is only applicable to ATMPs, whereas the "Specials" Exemption covers all medical products including ATMPs (Mahalatchimy and Faulkner). The Specials products are produced in compliance with the GMP by manufacturers granted by the Licensing Authority and their use is not limited to hospitals. The Specials products can be prescribed by doctors or dentists, supplementary prescribers (such as an appropriately qualified nurse or pharmacist), and others, and this differs from market authorization (Medicines and Healthcare products Regulatory Agency, 2014). As a Hospital Exemption is granted for ATMPs with no demonstrated safety and efficacy performance in some EU countries, there may be concerns about the safety and efficacy of the product, which may not have been confirmed (Cuende *et al.*, 2022). A warning against the use of unproven cell therapies were issued by the EMA in 2020 (EMA/CAT, 2020). Therefore, although Hospital Exemptions and Specials humanely provide patients with opportunities to access ATMPs, these systems should not be used to just avoid the high costs of a clinical trial.

Guidelines for quality, safety, and efficacy evaluation

In addition to the general guidelines for human cell-based medicinal products, a reflection paper on specific aspects related to marketing authorization applications for stem cell-based medicinal products has been issued by the EU (Table 1. #10 and #12) (EMA/CHMP, 2008a; EMA/CAT, 2011). The points to consider for quality (including manufacturing) as well as non-clinical and clinical studies are described in this reflection paper. In particular, the tumorigenicity of undifferentiated PSCs is mentioned in the sections on "Quality Considerations" and "Non-Clinical Considerations". In addition, information on the *in vivo* fate of ATMPs in clinical settings is included in the section on "Pharmacokinetics", which encourages the development and validation of new, non-invasive methods for tracking cells during clinical trials. On the assumption that ATMPs become part of the patient's body, unlike conventional drugs and medical devices, EU regulations focus on follow-up and risk management to determine the efficacy of ATMPs and their associated adverse effects. Therefore, the applicant

is required to provide details about follow-up and post-marketing surveillance, in addition to risk management plan. The "Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products" issued in the EMA provides details for the post-marketing follow-up and risk management of ATMPs (Table 1. #11) (EMA/CHMP, 2008b).

For ATMPs derived from human cells and tissues, it is necessary to comply with Directive 2004/23/EC (The European Parliament and the Council of the European Union, 2004) with respect to donors and processes such as cell preparation/processing, storage, and transfer. In addition, the guidelines on GMP for ATMPs were established to regulate the manufacturing and quality control specific to ATMPs in the EU (Table 1. #15) (European Commission, 2017). In accordance with the GMP, the WHO and ISO have also produced international guidance, the Technical Report Series 1044 - 56th report of the WHO Expert Committee on Specifications for Pharmaceutical Preparation (World Health Organization, 2022a), and ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories (International Organization for Standardization, 2017), respectively.

Japan

Product classification in Japan

Under The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices (Pharmaceuticals and Medical Devices Act, PMD Act), which regulates products intended for marketing, cell-processed products and products for gene therapy are collectively referred to as "regenerative medical products", which is a product type that is independent from that of drugs and medical devices. Products composed of living human or animal cells are classified as regenerative medical products in accordance with the presence or absence of cell processing and non-homologous use. Unlike in the US and the EU (and UK), the genetic modification of cells is considered to be a type of cell processing; therefore, genetically engineered cells for *ex vivo* gene therapy; for example, CAR T-cell products are classified as cell-processed products in Japan and not as products for gene therapy. PSC-derived products are also classified into cell-processed products. Under The Act for the Safety of Regenerative Medicine (RM Safety Act or ASRM), which is applied to regenerative/cellular therapies conducted as medical practice solely under the discretion of medical practitioners or as non-commercial clinical research, human or animal cells that are cultured or otherwise processed and are

not classified as regenerative medical products under the PMD Act are called “specified processed cells”.

Commercial clinical trials and non-commercial clinical studies

As described above, in Japan, the law applicable to CTPs with manufacturing/marketing authorization or commercial clinical trials for the purpose of obtaining manufacturing/marketing authorization differs from that applicable to medical practices using specified processed cells (namely, CTPs without manufacturing/marketing authorization) solely at the discretion of the medical practitioners or to non-commercial clinical studies on specified processed cells. Commercial clinical trials are regulated by the PMD Act and must be conducted in compliance with the GCP. If an application for marketing authorization is submitted, it is reviewed by the Pharmaceuticals and Medical Devices Agency (PMDA) and the Ministry of Health, Labour and Welfare (MHLW). In contrast, medical practices using specified processed cells based solely on physicians’ discretion and non-commercial clinical studies on specified processed cells are out of the scope of the PMD Act, and do not need to meet the data quality assurance-related requirements of the GCP. However, they must comply with the same ethics-related requirements as the GCP under the regulations of the RM Safety Act. Medical practices using specified processed cells have three classes in terms of the risk not conducting an appropriate safety assessment. Medical practices using PSC-derived specified processed cells are classified into “Class 1 Regenerative Medicine,” which are deemed the most difficult to assess for safety. Although the MHLW may provide opinions about the plans for non-commercial clinical studies on PSC-derived specified processed cells under the RM Safety Act, the reviews are not conducted by the PMDA but by institutional committees called Specified Accredited Committees for Regenerative Medicine. The RM Safety Act also provides the standards for institutions and cell processing facilities to ensure the safety of regenerative medicine and cell therapy.

Manufacturing/marketing authorization in Japan

In the process of manufacturing/marketing authorization for regenerative medical products, there is a conditional and term-limited approval system that allows for early patients’ access, in addition to the regular approval system. Based on the application data, PMDA and MHLW (not a pharmaceutical company) determine whether a term-limited approval system is applied to the regenerative medical product. In a case in which data about the efficacy, safety, and quality of CTPs are fully demonstrated, the CTPs can be approved via the regular

approval system. In the conditional and term-limited approval system, the MHLW may grant approval with necessary conditions that ensure its proper use and a term limit not exceeding seven years, if the following criteria are met: 1) The regenerative medical product pertaining to the application is not homogeneous; 2) The product is presumed to have the efficacy pertaining to the application; 3) The product is presumed to have value for use as a regenerative medical product because it does not have a significantly harmful effect in comparison with the efficacy, effectiveness or performance pertaining to the application. For regenerative medical products granted conditional and term-limited approval, it is necessary to plan and conduct a post-marketing study to verify the efficacy and safety and to re-submit the application to obtain official approval within seven years; this process differs from that of the regular approval system in Japan. Although any regenerative medical products granted conditional and term-limited approval are covered by public medical insurance, the data need to be evaluated for all patients using the product in question. It should be noted that it is impossible to conduct a randomized controlled study after marketing. Therefore, MHLW encourages that post-marketing data be consolidated in a nation-wide database called National Regenerative Medicine Database (NRMD), which was established by PMDA and has been operated by the Japanese Society for Regenerative Medicine (Okada *et al.*, 2018), to be evaluated with the cooperation of relevant academic societies (MHLW/PFSB/ELD, 2017).

Guidelines for quality, safety, and efficacy evaluation

In Japan, various notifications have been issued on CTPs, and their general descriptions are similar to the guidelines used in the US and the EU. To make it easier for developers, the MHLW has issued five guideline documents that describe the basic technical requirements for ensuring the quality and safety of CTP for each type of starting cell. Three of the five documents specialize in PSC-derived CTPs (Table 1. #17–19) (MHLW/PFSB, 2012a-c; Hayakawa *et al.*, 2015a-c). These three guidelines have a common basic content, although the allogeneic guidelines emphasize HLA typing and viral and bacterial infections associated with allogeneic PSCs. Understanding the genetic background of the donor is important when establishing allogeneic iPSC lines and may become much clearer when there is an understood hPSC-based product type. The difference between the guidelines for allogeneic iPSCs and ESCs is that, as ESCs are generated from the inner cell mass of a blastocyst, the guideline for ESCs describe ethical considerations, eligibility of the person or medical institution that collects the cells, and the validity of the method used to prepare fertilized embryos. It is also

necessary to understand the genetic background when establishing ESC lines, but this applies to male and female donors for producing *in vitro* fertilized embryos. Recently, for products whose clinical application or marketing are anticipated, evaluation guidelines have been issued as monographs based on the characteristics of the products, which are designed to encourage product developers and regulatory reviewers to share points to consider for discussions. (Table 1. #20, #21, #23, #25 and #27) (MHLW/PFSB/ELD/OMDE, 2013; MHLW/PFSB/ELD/OMDE, 2014; MHLW/PSEHB/MDED, 2016a; MHLW/PSEHB/MDED, 2021; MHLW/PSEHB/MDED, 2023).

As a ministerial ordinance on the standards for manufacturing and quality controls of regenerative medical products, the Good gene, Cellular, and Tissue-based products manufacturing Practice (GCTP) ordinance was issued (MHLW, 2014). The GCTP ordinance primarily considers structural facilities, manufacturing controls, and quality control. Quality risk management, verification, and product quality review have been stipulated in the GCTP, but not in the GMP. In general, validation is defined as an action of proving and documenting that any process, procedure or method actually and consistently leads to the expected results (World Health Organization, 2022). Validation is required for the manufacturing of pharmaceutical products; however, it is difficult to identify factors causing variations in cell preparation. In this case, the achievement of expected quality should be verified for each production batch by reviewing the written procedures, protocols, records, and reports, which be documented. This is called verification. In Japan, non-commercial clinical studies and medical practices conducted under the RM Safety Act also need to be compliant with the similar GCTP for the manufacturing and quality controls of specified processed cells.

A variety of biological materials can be used to manufacture regenerative medical products. In Japan, the Standards for Biological Raw Materials was established in 2003 (MHLW, 2018), which apply to biological raw materials derived from animals and human used for manufacturing of CTPs as well as to pharmaceutical products. With respect to the terminology used for materials employed in manufacturing in the EU, raw materials are considered to be the materials used during the manufacture of the active substance (e.g. culture media, growth factors) and they are not intended to form part of the active substance for ATMP (The European Parliament and the Council of the European Union, 2001b). The materials forming an integral part of the active substances are considered to be “starting materials”. The positioning of a starting material varies depending on the nature of the product. In the United State Pharmacopeia, raw materials are defined as all

444 materials used in the manufacture of cell and gene therapy products (such as cells, tissues, matrices, media, and
445 buffers), whereas ancillary materials are a subset of raw materials that come in contact with the cell or tissue
446 product, but they are not intended to be part of the final product. Therefore, the terminology used to describe
447 materials for CTP manufacturing is confusing because of the inconsistent classification, naming, and labeling
448 of intended use between regions (Solomon *et al.*, 2016). Biological manufacturing-related materials are
449 considered potential hazards (risk factors) in the following two cases: 1) when biological manufacturing-related
450 materials are present or remain in the final product in a significant amount that may cause undesirable health
451 problems in patients and 2) when there is a possibility (including a theoretical possibility) that biological
452 manufacturing-related materials may be contaminated with viruses or prions that may cause undesirable health
453 problems in patients administered the final products. On the other hand, products that are already approved as
454 pharmaceuticals or additives listed in Japanese official standards such as the Japanese Pharmacopoeia and the
455 Standards for Biological Raw Materials, and fractionated plasma products derived from domestic blood donors
456 and their equivalents are generally not considered hazards and are not subject to evaluation unless the approved
457 dosage is exceeded. The Standards for Biological Raw Materials require a great deal of information, including
458 donor screening, viral safety test results, and donor traceability, which are linked to issues with infectious agents.
459 However, in many cases, manufacturers of raw materials are often unable to provide full information about their
460 quality because some raw materials are used only for research purposes. In addition, the appropriate information
461 may not have been gathered for existing batches, or it is too difficult and costly to establish and the raw material
462 manufacturer has no commercial inducement. For example, their main income may be derived from supplying
463 their product for other purposes, such as research or food production; in this respect, hydrolysates used in culture
464 media are the by-product of dairy-based foods. To improve such issues associated with biological raw materials,
465 it may be beneficial to implement the following: 1) collaboration with the raw material producer to establish a
466 type of "master drug file" for the material, 2) review the necessity for additional product or raw material testing
467 regimes based on a description of the manufacturing process and raw materials, and their origins, and 3) conduct
468 a site inspection of the raw material producer.

469 A case-by-case approach is required to develop CTPs, resulting in no uniform standards and many
470 uncertainties. With this background, the PMDA has introduced the current approach to quality control, non-
471 clinical safety evaluation, and points to consider in clinical studies (Table 1. #22) (MHLW/PSEHB/MDED,

2016b) based on its experience in face-to-face consultation and review concerning CTPs with characteristics that are significantly different from those of pharmaceuticals and medical devices.

Transformed cells and residual undifferentiated PSCs as product impurities are potential tumorigenic hazards specific for CTPs. A guideline has been issued that propose representative examples of testing methods for detecting undifferentiated PSCs and transformed cells intermingled with human CTPs as well as points to consider when selecting testing methods for evaluating the quality and safety of particular human CTPs (Table 1. #24) (MHLW/PSEHB/MDED, 2019). In this guidance document, it is noteworthy that the section of tumorigenicity-related tests for human ESC/iPSC-based products is separated into three parts, which depends on the purpose of testing for CTPs; 1) quality control of cell substrates, 2) quality control of intermediate/final products during manufacturing processes, and 3) non-clinical safety assessment of final products. On the other hand, another guidance document provides several points to consider for the tumorigenicity assessment of unlicensed PSC-derived processed cells in non-commercial clinical studies, which are regulated by the RM Safety Act (Table 1. #26) (MHLW/HPB/RDD, 2021). This document states that to provide patients with therapies using PSC-derived processed cells as safely and rapidly as possible, it is necessary to collect and accumulate scientific data in non-commercial clinical studies for future development of regenerative medicine and cell therapy; particularly those that accumulate data on the genomic instability of products and the technical issues associated with testing methods.

Global technical standardization and regulatory harmonization/convergence

Regulations for CTPs have not yet been harmonized. Discussions on standardization and regulatory harmonization/convergence are ongoing in various consortia involving members from industry, academia, and regulations [Fig. 1]. The following is an overview of the scope and efforts of representative consortia.

Standardization of PSCs as raw materials for CTPs

The International Stem Cell Initiative

In the 2000s, human PSCs were new and their properties were not yet fully understood. In addition, the results reported by laboratories often differed. Recognizing these challenges, researchers organized the first meeting of the International Stem Cell Forum (ISCF; later renamed the International Stem Cell Foundation) in

Paris in 2003 (https://www.timeshighereducation.com/news/12-nations-unite-to-advance-stem-cell-research/178156.article). Twenty-two countries participated in the Forum, including the US, the UK, France, Germany, and Japan, and proposed the launch of a project for collecting and comparing PSCs from around the world, with the aim of determining the common characteristics of human PSCs. Funded by the ISCF, the International Stem Cell Initiative (ISCI) was initiated in the autumn of 2003 with the goal of sharing knowledge about the characteristics of human PSCs and stimulating the development of their applications (Andrews *et al.*, 2005). To date, the ISCI has compared the expression of undifferentiated and differentiated markers in the undifferentiated state and in the embryoid state of a number of human PSC lines (ISCI-1) (International Stem Cell Initiative, 2007), examined their profile of genomic variation by long-term *in vitro* culture (ISCI-2) (International Stem Cell Initiative Consortium, 2010; Närvä, *et al.*, 2010), and evaluated the performances of methods to measure pluripotency and differentiation potential of human PSCs (ISCI-3) (International Stem Cell Initiative, 2018). In addition, the ISCI Steering Committee has recently published a collective perspective on the current understanding of the genetic and epigenetic variations that occur in human PSCs (Andrews, *et al.*, 2022).

The International Stem Cell Banking Initiative

Based on the results of the ISCI-1 activities, the International Stem Cell Banking Initiative (ISCBI, www.iscbi.org) was launched in 2007, which consists of researchers from stem cell banks from 28 countries around the world, including the US, France, Germany, Japan, and others, with the UK Stem Cell Bank as its core. The ISCBI supports the international technical standardization of PSCs through discussions on cell line procurement, cell banking systems, optimization of storage methods, advanced aseptic processing, and quality control systems, including virus testing (International Stem Cell Banking Initiative). Reports and results from ISCBI activities have been published in papers and other media (International Stem Cell Banking Initiative, 2009; Crook *et al.*, 2010; Andrews *et al.*, 2015; Stacey & Healy 2021; Kim *et al.*, 2022).

Pluripotent Stem Cell Standards Initiative Task Force on the International Society for Stem Cell Research

The International Society for Stem Cell Research (ISSCR) is a non-profit stem cell research society that holds a forum for sharing information on PSCs in collaboration with stem cell societies in various countries. The

Pluripotent Stem Cell Standards Initiative Task Force of the ISSCR summarizes information on the quality of PSCs that is recommended when submitting papers to scientific journals, based on the evaluation of cell line characteristics. The project has drafted a guidance document for basic and genomic characterization of PSCs, identifying undifferentiated stem cells, assaying pluripotency, and stem cell-based model systems, which will be finalized and published in the first half of 2023 (International Society for Stem Cell Research).

The Global Alliance for iPSC Therapies

The Global Alliance for iPSC Therapies (GAiT) is an international consortium of interested partners some of which are resource centers that facilitates the therapeutic use of immunogenetically matched and clinical-grade iPSCs for the benefit of patients worldwide. The scope of GAiT is to achieve a consensus on donor selection, screening criteria, manufacturing, and quality parameters, and to gain agreement on the quality standards with regulators. In addition, GAiT is involved in the development of an external quality assurance scheme for iPSC lines intended for clinical use (Sullivan *et al.*, 2020).

Human Pluripotent Stem Cell Registry

The Human Pluripotent Stem Cell Registry (hPSCreg) is an international resource that registers and collects the standard properties of hPSC lines (Mah *et al.*, 2020). The hPSCreg platform was established to provide a database for clinical studies involving PSC-derived products (Kobold *et al.*, 2020); the database acts a regulatory tool of the EC and ensures that all iPSC lines used in EC-funded research are ethically sourced and available for public research.

ISO TC276 Working Group 2

The ISO establishes international definitions, standards, and guidelines. TCs has been established for discussing a variety of items in the ISO. Regenerative medicine is discussed in TC276: Biotechnology, which is divided into five working groups (WG1: Terminology, WG2: Biobanking, WG3: Analytical Methods, WG4: Bioprocessing, and WG5: Data Processing and Integration). Other relevant TCs include ISO/TC 150/SC 7 – tissue-engineered medical products, which discusses the standardization of methods for evaluating the efficacy of regenerative medicine products in combination with scaffolds; ISO/TC 194/SC 1 – tissue product safety,

556 which discusses biological evaluation methods for the safety of medical materials and devices; and ISO/TC
557 198/WG9 – aseptic processing, which discusses the processes of aseptic manipulation for regenerative medicine
558 products.
559 ISO/TC 276/WG2 discusses standardizations related to biobanks and bioresources, including human cells,
560 animal cells, plants and seeds, and bacteria. Standards of PSC stocks used in research are pursued in WG2 and
561 these were recently published as ISO 24603:2022 (International Organization for Standardization, 2022a).
562 One of the future goals of WG2 is to standardize PSC stocks for clinical use.
563
564 *Standardization of technologies for CTPs*
565 *ISO: TC276 WG1/WG3/WG4*
566 Working groups 1, 3, and 4 in ISO TC276 are related to technologies for CTPs. In WG1, terminology is
567 defined for use as a common language among countries in relation to trade. In WG3, analytical methods for
568 measurements related to cells and nucleic acids are standardized. Discussions have been held on available
569 indicators for accurately evaluating equipment and cell counting methods that are difficult for SI-traceable
570 measurement, as well as the requirements and measurement techniques used to identify cells. The documents
571 already published include the following: "ISO 23033:2021 Biotechnology — Analytical methods — general
572 requirements and considerations for the testing and characterization of cellular therapeutic products," which
573 describes the general approach used to determine methods for evaluating the quality of cell therapy products
574 and considerations for determining the checklist quality of CTPs (International Organization for
575 Standardization, 2021a), and "ISO 20391-1:2018 Biotechnology – cell counting –Part 1: general guidance
576 on cell counting methods," which describes cell counting methods (International Organization for
577 Standardization, 2018). WG4 standardizes bioprocessing, and this is particularly relevant for regenerative
578 medicine. This WG has worked on the standardization of raw materials, transportation, equipment, and
579 stabilization of cell manufacturing, and the following documents have been published: ISO 21973:2020
580 Biotechnology – general requirements for the transportation of cells for therapeutic use; ISO/TS 23565:2021
581 Biotechnology – bioprocessing – general requirements and considerations for equipment systems used in the
582 manufacturing of cells for therapeutic use; ISO 20399:2022 Biotechnology — Ancillary materials present
583 during the production of cellular therapeutic products and gene therapy products (International Organization

for Standardization, 2020;2021b;2022b)

In addition, documents associated with 18 other topics are currently being developed in ISO 276 (International Organization for Standardization). For example, "ISO/WD 18162 Biotechnology – Biobanking – Requirements for human neural stem cells derived from pluripotent stem cells," which is the standard for biobanking of iPSC-derived hNSCs used for research and development in the life science field, but not for *in vivo* human use, clinical use, or therapeutic purposes, and "ISO/CD 8472-1 Biotechnology – Data interoperability for stem cell data – Part 1: Framework" and "ISO/AWI 8472-2 Biotechnology – Data interoperability for stem cell data – Part 2: Key characteristics of stem cell data" which are standards that provide a framework for data interoperability of stem cells that are being developed.

The Standards Coordinating Body for gene, cell and regenerative medicines and cell-based drug discovery

The Standards Coordinating Body for gene, cell and regenerative medicines and cell-based drug discovery (SCB) is a non-profit organization, which was established in the US at an initiative of the Alliance for Regenerative Medicine (ARM) and other regenerative medicine stakeholders and industry to promote standardization in the nascent regenerative medicine industry. The long-term goal of the SCB is to support efficient and effective product development and review by assisting in the development of standards available for regulatory review and improving the cost, time, and resources for product development and approval (Standards Coordinating Body). The SCB provides coordination to promote the development of standards for regenerative medicine manufacture, and it continues to participate in ISO TC276 discussions, as well as FDA (CBER) and NIST.

Global regulatory harmonization/convergence

International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use

The objectives of ICH are: 1) to ensure that patients have timely and continuous access to new pharmaceutical products; 2) to avoid unnecessary duplication of clinical studies in humans; 3) to ensure efficient development, registration, and manufacturing of safe, effective, and high-quality pharmaceutical products; and 4) to promote public health by facilitating international harmonization of technical requirements that help reduce animal testing without compromising safety and efficacy. The ICH guidelines cover all aspects of pharmaceutical

products, including ICH Q (1–14) for quality, ICH S (1–12) for non-clinical (safety), ICH E (1–20) for clinical (efficacy), and ICH M (1–14) for multidisciplinary evaluation. Of these guidelines, Q5A, Q5D, Q5E, Q6B, and S6, while CTPs are not officially within their scopes, are those that provide very useful information about CTP development and manufacturing. However, it is difficult to uniformly apply them to CTPs, as in the case with conventional medicines. At the 12th Summit of Heads of Medicines Regulatory Agencies in 2017, recognizing the need to implement regulations that appropriately reflect the characteristics of regenerative medicine products and their international harmonization, an agreement was made to promote international regulatory harmonization for regenerative medicine products by utilizing existing international frameworks (WHO, ICH, *etc.*). However, no global regulatory guidelines have been established for the use of CTPs to date.

World Health Organization

The World Health Organization (WHO) is an organization that was established to help countries cooperate with each other to promote and protect the health of all people and has a role in the standardization of biologicals via its Expert Committee on Biological Standardization (ECBS). The WHO ECBS has currently progressed beyond well-established biologicals to cell-based medicines. With the aim of explaining WHO's current thinking on the regulation of cellular and gene therapy products, promoting convergence, and encouraging member states to strengthen their regulatory systems for the regulation of cellular and gene therapy products, WHO recently adopted the "WHO approach towards the development of a global regulatory framework for cell and gene therapy products" (World Health Organization, 2022b). This document is not intended to be a comprehensive overview of the regulatory requirements for cellular and gene therapy products or the different regulatory frameworks that currently exist in different jurisdictions. The purpose of this document is to outline some of the basic principles that are important for appropriate regulatory oversight for different types of cellular and gene therapy products. In the future, WHO will develop more comprehensive written guidance on specific topics relevant to the regulation of cellular and gene therapy products, as needed.

Other platforms

The International Alliance for Biological Standardization

The International Alliance for Biological Standardization (IABS) was founded in 1955 to improve the quality

and regulation of biological products from human and animal origin in association with the WHO, manufacturers, and regulatory bodies. The IABS consists of members in over 50 countries (International Alliance for Biological Standardization), and meetings have taken place in 2014, 2016, 2018, and 2020. Notably, the meeting in 2018 meeting was focused on the manufacture of human pluripotent stem cell-based products (Abbot *et al.*, 2018). The IABS collaborated with the WHO to publish a white paper on scientific considerations when evaluating cell therapy products (Petricciani *et al.*, 2017).

The Cell Therapy-Tracking, Circulation, & Safety Committee, and the Health and Environmental Sciences Institute

The Health and Environmental Sciences Institute (HESI) functions as a global platform for industry, academia, and government to engage in scientific discussions on methods that evaluate the quality and safety. Methods to evaluate the tumorigenicity of CTPs and analyze their biodistribution have been studied by the Cell Therapy-TRAcking, Circulation, & Safety (CT-TRACS) Committee. In Japan, the Japan Agency for Medical Research and Development (AMED) has been supporting a public-private partnership initiative for the multisite evaluation study on analytical methods for non-clinical safety assessment of human-derived regenerative medical products (MEASURE), which aims to validate methods for assessing the tumorigenicity of CTPs. The data from the MEASURE project are shared with HESI CT-TRACS members to discuss the validity and points to consider for the test methods. Their cooperation is expected to be helpful for the international standardization and regulatory harmonization/convergence of test methods for CTPs in the future (Sato *et al.*, 2019; Kamiyama *et al.*, 2021; Watanabe *et al.*, 2021).

Conclusion

In this paper, we introduce regulations related to PSC-derived CTPs, mainly those of the US, EU, and Japan, and the efforts made to standardize various regulatory platforms (Fig. 1 and Table 2). In summary, PSC-derived CTPs are currently evaluated using the general approach applied to CTPs. In the US, although the FDA's Advisory Committee has provided points to consider for preclinical safety testing and patient monitoring for ESC-derived CTPs, no official guidelines have been issued and thus far, CTPs derived from stem cells are reviewed within the framework of 351 HCT/Ps. Conversely, in the EU and especially in Japan, guidelines for

668 CTPs derived from stem cells have been specifically issued, addressing tumorigenicity, one of the concerns with
669 PSC-derived CTPs. Refer to hESC guidelines available for the development of CTPs in the UK, the UK Stem
670 Cell Bank was established in 2003 to facilitate the sharing and use of quality controlled human stem cell lines
671 by clinical and research communities. UK documentation on the use of hESCs was published by the "Steering
672 Committee for the UK Stem Cell Bank and the Use of Human Embryonic Stem Cells", which provides guidance
673 and assistance on best practice to those working with stem cell lines (UK Stem Cell Bank steering committee,
674 2010).

675 As described above, tumor formation of residual tumorigenic cells is a concern for the clinical application
676 of PSC-derived products. However, the threshold levels for residual tumorigenic cells, including
677 undifferentiated hiPSC, in tumor formation attributed to CTPs are ill-defined. As the properties of CTPs and the
678 performance of testing methods determine the threshold, the possibility of tumor formation from a single
679 tumorigenic cell cannot be completely excluded. In practice, a 50% Tumor Producing Dose (TPD_{50}) from
680 positive control iPSCs was reported as 132 and 631 cells under the condition that is suitable for iPSC survival
681 using *in vivo* testing with severely immunodeficient NOG mice (Kanemura *et al.*, 2014; Yasuda *et al.*, 2018).
682 Therefore, specifications for residual tumorigenic cells in CTPs should be defined as being "less than the
683 detection limit using the testing method" unless justified otherwise. *In vivo* testing would also be required to
684 evaluate the tumorigenicity of transplanted CTPs in the microenvironment, especially for PSC-derived products
685 and other types of CTPs with limited clinical experience. In such a case, the selection of animal models and
686 species requires scientific justification. The use of genetically immunodeficient animals and humanized animals
687 is recommended in the US and EU to monitor tumor formation over the long-term (Table 1. #5 and #12)
688 (FDA/CBER, 2013; EMA/CAT, 2011). In Japan, the use of severely immunodeficient NOG mice and NSG
689 mice is preferred to that of nude mice, with respect to the easy engraftment of xenogeneic cells (Table 1. #24)
690 (MHLW/PSEHB/MDED, 2019). Also, several *in vitro* tests have shown to be comparable to *in vivo* tests using
691 such severely immunodeficient mice at least in terms of the detection limit for tumorigenic cellular impurities
692 in intermediate or final products. The FDA Modernization Act 2.0 was finally approved in the US, and this aims
693 to improve the process involved in approving drugs and commits to significantly reducing the use of animals in
694 laboratory testing (FDA Modernization Act 2.0, 2022). Therefore, clinical trial leaders will use animal trial
695 alternatives instead of traditional animal modeling for drug development in nonclinical studies. In the EU, the

696 document “Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement)
697 testing approaches” has already been issued by the EMA (EMA/CHMP/CVMP, 2012). In this context, as
698 critical quality attributes of PSC-derived CTPs associated with *in vivo* tumor formation are also gradually
699 revealed, *in vitro* testing would be more useful and reasonable for tumorigenicity evaluation, as this would
700 reduce the number of animals used in *in vivo* tumorigenicity testing in the future.
701 In the regulations of the three (or four including UK) ICH founding states, the risk-based approach as a
702 regulatory principle is more emphasized for CTP than for other drugs. Each country or region has developed its
703 own expedited approval process for CTPs that differs from that for conventional pharmaceuticals. Some of these
704 systems allow patients early access to CTPs when safety is assured, and putative efficacy is confirmed. Although
705 they require continuous evaluation, such as post-marketing surveillance, these programs can address the needs
706 of patients with diseases for which no other treatments are available and promote product development. Various
707 consortia have recently discussed the move toward standardization of CTPs regulation in accordance with their
708 respective scopes. Indeed, the US is focusing on standardization as a national policy, so the importance of
709 standardization is expected to increase in the future. Standardization would be beneficial for both CTP
710 development and regulation, which would promote their clinical application. Patients would have early access
711 to CTPs if standardization resolved issues related to drug lag. The ICH is responsible for the harmonization of
712 pharmaceutical regulations, but it has not yet actively worked towards obtaining regulatory
713 harmonization/convergence of CTPs. Hopefully, a consensus on regulations related to CTPs, including PSC-
714 derived products, will be formed through the ICH in the future. As the final products of PSC-derived CTPs are
715 diverse, we suggest that the commonalities and differences between the quality properties of products should
716 be defined, and this will require a flexible approach to issues demonstrated by scientific findings. It is necessary
717 to establish a system for sharing data and their accompanying interpretations and perceptions on a global scale
718 as data related to PSC-derived CTPs are accumulated.
719

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Author contributions

Y.S. and A.U. provided conception and obtained funding for this paper; T.H. surveyed the updates of regulations and consortia related to CTPs and wrote the first draft in collaboration with S.Y. All authors reviewed the manuscript and approved its final version.

Declarations of interests

The authors declare no competing interests.

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Figure legend

Figure 1 Platforms for discussions on international regulatory harmonization/convergence and technical standardization of cell-based therapeutic products

Representative platforms/consortia related to international regulatory harmonization/convergence and technical standardization of pluripotent stem cell-based therapeutic products are shown (open, no discussion on international harmonization and standardization; filled, discussion on international harmonization and standardization).

Industry: ISO, International Organization for Standardization; ASTMi, American Society for Testing and Materials International; BSI, British Standards Institution; SCB, Standards Coordinating Body for Cellular/Gene and Regenerative Therapies.

Academia: ISCT, International Society for Cell & Gene Therapy; ISSCR, International Society for Stem Cell Research; GAiT, Global Alliance for iPSC Therapies; ISCI, International Stem Cell Initiative; ISCBI, International Stem Cell Banking Initiative; ISCF, International Stem Cell Forum/Foundation; DIA, Drug Information Association; CASSS, California Separation Science Society.

Regulatory: FDA, Food and Drug Administration; EMA, European Medicines Agency; PMDA, Pharmaceuticals and Medical Devices Agency; MHLW, Ministry of Health, Labour and Welfare; ICH, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; IPRP, International Pharmaceutical Regulators Programme; WHO, World Health Organization.

Others: HESI CT-TRACS, Committee for Cell Therapy-Tracking, Circulation & Safety, Health and Environmental Sciences Institute; IABS, International Alliance for Biological Standardization; DIA, Drug Information Association; CASSS, California Separation Science Society; RAPS, Regulatory Affairs Professional Society; PDA, Parenteral Drug Association.

1149 **Table 1 Regulatory guidelines related to cell-based therapeutic products**

Agency	Guidelines	Relevant laws and regulations
FDA	1. Proposed approach to regulation of cellular and tissue-based products (docket number 97N-0068). 2. Eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (docket number 2004D-0193). 3. Potency tests for cellular and gene therapy products (docket number FDA-2008-D-0520). **4. CTGTAC. Cellular therapies derived from human embryonic stem cells: considerations for preclinical safety testing and patient monitoring. Meeting # 45 (briefing document, not guidance for industry).** 5. Preclinical assessment of investigational cellular and gene therapy products (docket number FDA-2012-D-1038). 6. Considerations for the design of early-phase clinical trials of cellular and gene therapy products (docket Number FDA-2013-D-0576). 7. Regulatory considerations for human cells, tissues, and cellular and tissue-based products: Minimal manipulation and homologous use (docket number FDA-2017-D-6146). 8. Regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps) – Small entity compliance guide (docket number: FDA-2022-D-0563) 9. Voluntary consensus standards recognition program for regenerative medicine therapies (docket number: FDA-2022-D-0745)	a. Federal Food, Drug, and Cosmetic Act b. Public Health Service Act c. 21st Century Cures Act (Public Law 114-255) d. Code of Federal Regulations Title 21 (21CFR) Part 1271
EMA	10. Guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006). 11. Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products (EMEA/149995/2008). **12. Reflection paper on stem cell-based medicinal products (EMA/CAT/571134/2009).** 13. Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced Therapy Medicinal Products (EMA/CAT/CPWP/686637/2011). 14. Reflection paper on a proposal to enhance early dialogue to facilitate accelerated assessment of priority medicines (PRIME) Draft (EMA/CHMP/57760/2015). 15. Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products [C(2017) 7694 final]. 16. Guideline on Good Clinical Practice specific to Advanced Therapy Medicinal Products [C(2019) 7140 final].	d. REGULATION (EC) No 1394/2007 on Advanced Therapy Medicinal Products e. Directive 2009/120/EC
MHLW	**17. Guidelines on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of autologous human iPS(-like) cells. (Notifications No. 0907-4, PFSB) (2012).** **18. Guidelines on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human iPS(-like) cells. (Notifications No. 0907-5, PFSB) (2012).** **19. Guidelines on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human ES cells. (Notifications No. 0907-6, PFSB) (2012).** **20. Evaluation guidelines for autologous iPS cell-derived retinal pigment epithelial cells. (Notification No. 0529-1, Attachment 1, PFSB/ELD/OMDE) (2013)** **21. Evaluation guidelines for allogeneic iPS cell-derived retinal pigment epithelial cells. (Notification No. 0912-2, Attachment 1, PFSB/ELD/OMDE) (2014)** 22. Technical guidance for quality, non-clinical and clinical studies of regenerative medicine products (human cell-processed products). (PSEHB/MDED Administrative Notice No. 0614043) (2016) **23. Evaluation guidelines for articular cartilage regeneration using allogeneic iPS (like) cell-processed products. (Notification No. 0630-1, Attachment 2, PSEHB/MDED) (2016)** **24. Points to consider regarding tests to detect undifferentiated pluripotent stem cells/transformed cells, tumorigenicity tests, and genomic stability evaluation for human cell-based therapeutic products. (Notification No. 0627-1, PSEHB/MDED) (2019)** **25. Evaluation guidelines for the treatment of (traumatic) subacute spinal cord injury using human (allogeneic) iPS (like) cell-processed products. (Notification No. 0226-1, PSEHB/MDED) (2021)** **26. Points for certified special committees for regenerative medicine to consider when evaluating tumorigenicity assessment in provision plans of regenerative medicine using human pluripotent stem cells. (Notifications No. 0309-1, HPB/RDD) (2021).** **27. Evaluation guidelines for the treatment of ischemia cardiomyopathy using human (allogeneic) iPS cell-derived cardiomyocyte cell-sheet. (Notification No. 0331-15, PSEHB/MDED) (2023)**	f. Pharmaceuticals and Medical Devices Act g. Act on the Safety of Regenerative Medicine

1150 **Bold:** Document with specific descriptions about pluripotent stem cell-derived products

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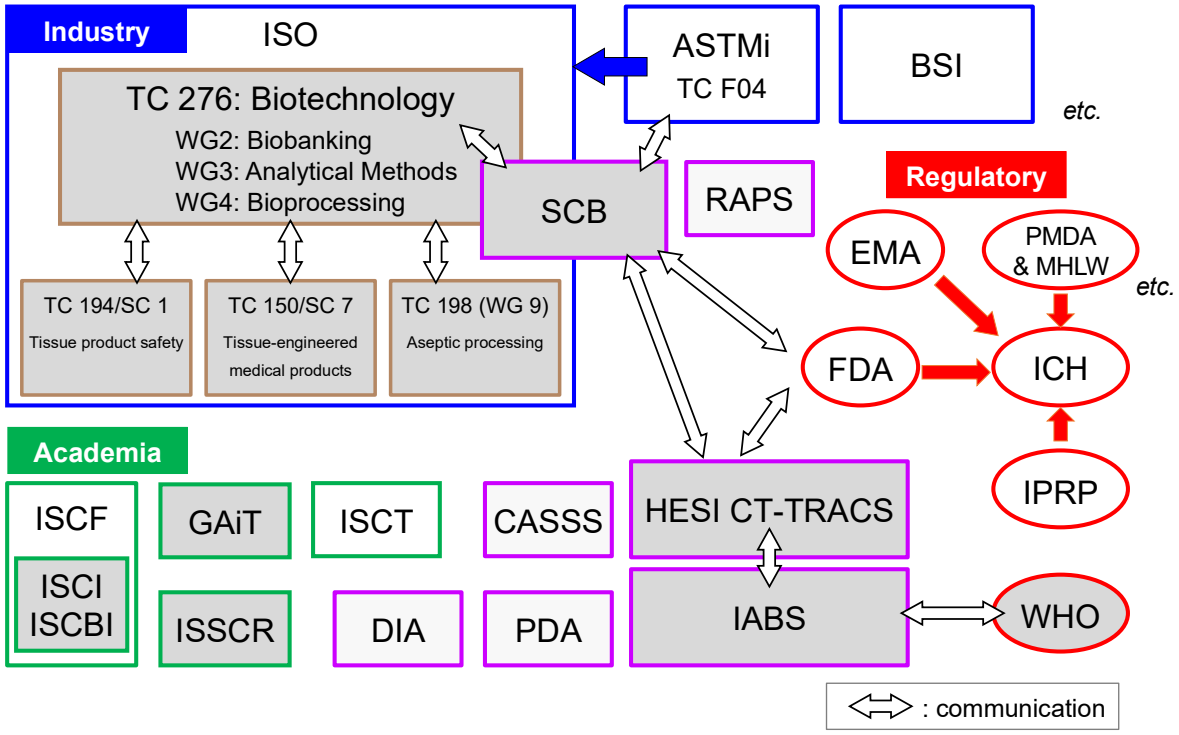
Table 2. Regulation of cell-based therapeutic products in the United States, the European Union [& the United Kingdom], and Japan

	US	EU [UK]	Japan
Classification of PSC-derived CTPs	351 HCT/P	Advanced Therapy Medicinal Product (ATMP)	Cell-Processed Product
Product Type	Biologics or Medical Devices	Medicinal Products	Regenerative Medical Products
Regulatory Authority	FDA	EMA (MHRA in UK)	MHLW and PMDA
Compliance with GCP in Clinical Trials	Essential	Essential	Essential in commercial clinical trials
Good Practice(s) for Quality and Manufacturing Controls	cGMP (for biologics) or QSR (for medical devices)	GMP for ATMPs	GCTP
Conditional Marketing Authorization with Putative Efficacy	RMAT / HDE	Hospital Exemption (Article 28 of Regulation 1394/2007/EC)	Conditional and Term-limited Approval
Use of Unlicensed Products	Federal regulations prohibit manufacturers from introducing unapproved 351 HCT/Ps into interstate commerce.	Specials (Article 5 (1) of Directive 2001/83/EC)	Specified Processed Cells under the RM Safety Act

Hirai T *et al.* Fig. 1



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