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Mechanism of action, potency and efficacy: considerations for cell therapies

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Abstract

One of the most challenging aspects of developing advanced cell therapy products (CTPs) is defining the mechanism of action (MOA), potency and efficacy of the product. This perspective examines these concepts and presents helpful ways to think about them through the lens of metrology. A logical framework for thinking about MOA, potency and efficacy is presented that is consistent with the existing regulatory guidelines, but also accommodates what has been learned from the 27 US FDA-approved CTPs. Available information regarding MOA, potency and efficacy for the 27 FDA-approved CTPs is reviewed to provide background and perspective. Potency process and efficacy process charts are introduced to clarify and illustrate the relationships between six key concepts: MOA, potency, potency test, efficacy, efficacy endpoint and efficacy endpoint test. Careful consideration of the meaning of these terms makes it easier to discuss the challenges of correlating potency test results with clinical outcomes and to understand how the relationships between the concepts can be misunderstood during development and clinical trials. Examples of how a product can be "potent but not efficacious" or "not potent but efficacious" are presented. Two example applications of the framework compare how MOA is assessed in cell cultures, animal models and human clinical trials and reveals the challenge of establishing MOA in humans. Lastly, important considerations for the development of potency tests for a CTP are discussed. These perspectives can help product developers set appropriate expectations for understanding a product's MOA and potency, avoid unrealistic assumptions and improve communication among team members during the development of CTPs.

Keywords Cell therapy product, Efficacy, Efficacy endpoint, Efficacy endpoint test, Mechanism of action, Potency, Potency test

Introduction

Challenges of MOA and potency

Determining the MOA and developing adequate potency tests remain challenging for CTPs [1–6]. US regulations require that CTPs have a potency test for licensure. Potency tests should be based on the product's MOA [7, 8]. The goal of a potency test is to assure that the product is able to achieve its intended mechanism of action. Other major roles of potency tests are to assess manufacturing consistency and product stability. Potency tests are often bioassays that involve measuring a response in cells.

For many of the 27 US FDA-approved CTPs (as of February 2024), the relationships between the potency tests and proposed MOAs are unclear. Table 1 highlights representative examples of the information regarding potency and MOA taken from the regulatory documentation for seven of the FDA-approved CTPs (see Supplementary File 1 for information about all 27 CTPs). The FDA has a useful website containing

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Table 1 Regulatory comments regarding potency & MOA For FDA-approved cell therapies

Product (year approved, company): Description	Indication	Potency test	MOA
Provenge (2011, Dendreon): Autologous cellular immunotherapy (CD54 + cells activated with PAP-GM-CSF)	Prostate cancer	Surface expression of CD54 (ICAM-1) on APCs after culture with PAP-GM-CSF (flow cytometry)	*Package Insert:* "While the precise **mechanism of action is unknown**, PROVENGE is designed to induce an immune response targeted against PAP, an antigen expressed in most prostate cancers."
Gintuit (2012, Organogenesis): Allogeneic cultured keratinocytes and fibroblasts in bovine collagen	Oral mucogingival defects	Histology with morphological assessments	*Package Insert:* "GINTUIT does not function as a tissue graft. The MOA by which GINTUIT increases keratinized tissue at the treated site has not been identified." *FDA SBRA:* "The histology assay is a good measure of the structural integrity of the product, however**, the assay is not an adequate, sensitive measure of biological activity**."
MACI (2016, Vericel): Autologous cultured chondrocytes on porcine collagen membrane	Knee cartilage defects	PCR measurement of aggrecan gene expression	*Package Insert:* "No clinical pharmacology studies have been conducted with MACI and a **mechanism of action has not been established**."
Kymriah (2017, Novartis): CD19-directed genetically modified autologous T-cell immunotherapy	B-cell acute lymphoblastic leukemia	Interferon-γ production by test article upon stimulation with CD19 + cells	*Package Insert:* "Upon binding to CD19-expressing cells, the CAR transmits a signal to promote T-cell expansion, activation, target cell elimination, and persistence of the KYMRIAH cells" *FDA Briefing Document:* "In the clinical trials, IFN-γ production varied greatly from lot-to-lot, **making it difficult** to correlate IFN-γ production with in vitro safety or efficacy."
Stratagraft (2021, Stratatech): Allogeneic cultured keratinocytes and dermal fibroblasts in murine collagen	Thermal burns	*Not reported*	*Package Insert:* "In vitro studies have shown that Stratagraft **secretes** human growth factors and cytokines and contains human ECM proteins."
Rethymic (2021, Enzyvant): Allogeneic processed thymus tissue	Athymia	Histology	*Package Insert:* "The **proposed** mechanism of action involves the migration of recipient T cell progenitors from the bone marrow to the implanted Rethymic slices, where they develop into naïve immunocompetent recipient T cells."
Lantidra (2023, CellTrans): Allogeneic pancreatic islets	Type 1 diabetes	i) insulin release in glucose stimulated islets; ii) islet yield by microscopy; iii) viability by microscopy	*Package Insert:* "The primary mechanism of action of LANTIDRA is **believed** to be secretion of insulin by infused (transplanted) β- cells."
Amtagvi (2024, Iovance): Autolologous T-cell therapy made of ex vivo-expanded lymphocytes harvested from patient tumors	Melanoma	i) redacted, ii) redacted, iii) redacted, iv) redacted, v) dose (total viable cells), vi) redacted and vii) redacted	*Package Insert:* ""The specific mechanism of action of AMTAGVI (lifileucel) is **unknown**."

FDA Food and Drug Administration, *PAP-GM-CSF* human prostatic acid phosphatase (PAP), an antigen expressed in prostate cancer tissue, linked to human granulocyte–macrophage colony-stimulating factor (GM-CSF), an immune cell activator, *SBRA* Summary Basis for Regulatory Action. Bolded text highlights areas of uncertainty or concern and bolding was added by the authors

Source: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

information for approved CTPs [9]. Two documents posted for each product are particularly useful: the "Product Insert" and "Summary Basis for Regulatory Action" (SBRA), which contain information about the clinical trials, product testing, potency and MOA. The regulatory documentation for Provenge, Gintuit, MACI and Amtagvi indicate that the MOA is not known for these products (Table 1). For Kymriah, the documentation states that it is difficult to correlate the potency test results with efficacy. The documentation for Stratagraft discusses data regarding the product activity but does not directly state whether this activity is to be regarded as the MOA. For Rethymic and Lantidra, the documentation uses the words "proposed" and "believed" when discussing the MOA to indicate uncertainty. The lack of clarity regarding potency and MOA lead to the question: "Why is there such a challenge with MOA and potency?" This perspective article attempts to shed light on this question.

Table 2 summarizes information on potency tests for the 27 approved CTPs. Supplementary File 1 summarizes the key aspects of each of the FDA-approved CTPs including product name, year approved, sponsor, product description, indication, clinical trial structure, efficacy endpoints, MOA, potency test, comments and references. This information was used as background for many of the points highlighted in this perspective.

Correlation of potency test with clinical outcome

It is desirable for the potency test to reflect clinical efficacy [7, 8]. Manufactured units that show high potency via the potency test should tend to show an efficacious benefit to patients, while units with lower potency, as measured by the potency test, should have a less efficacious benefit to patients. However, a correlation between the potency test and clinical efficacy is not required. If a product is efficacious and the risk–benefit profile is acceptable, then a product may receive regulatory marketing clearance even if the potency test does not correlate with efficacy endpoint test results (Table 1). For CTPs, MOAs may not be fully understood making it difficult to relate MOAs to potency or efficacy.

Kymriah: relationship between potency test results and clinical outcome

In 2017, Kymriah (tisagenlecleucel) was the first chimeric antigen receptor (CAR) T-cell therapy that was cleared for marketing by FDA. Kymriah is used to treat leukemia and potency was defined as the ability of the CAR T-cells to secrete interferon-γ (IFN-γ) following exposure to target cells expressing CD19. The FDA held a meeting of the Oncologic Drugs Advisory Committee to discuss this revolutionary technology in a public forum. The online documentation contained a noteworthy graph showing the relationship between potency measurements and efficacy endpoints (Fig. 1) [10]. To the authors' knowledge, this is the only publicly available data to show the relationship between potency testing results and clinical outcome for a US-approved CTP. The graph shows that the potency test results correlated with remission, but that there was overlap between responders and non-responders. The FDA Briefing Document from the meeting stated "In the clinical trials, IFN-γ production varied greatly from lot-to-lot, making it difficult to correlate IFN-γ production in vitro to tisagenlecleucel safety or efficacy" (Table 1) [11].

Definitions

Clear definitions are critical to improving the understanding of the relationships among MOA, potency and efficacy. Figure 2a provides definitions of 6 key terms and delineates their relationships as discussed below. Definitions for terms were adapted from existing definitions but may not be identical to definitions in the source documents. Definitions were created for terms that had not previously been defined.

Measurand and measurement

Since the current discussion is focused on a product attribute (potency) and how to measure it (potency test), it is important to understand the key metrological term, measurand.

Measurand: "*the quantity or property intended to be measured*" [12]

A measurand is the attribute or property of a material that is being assessed. A measurand and a material attribute can be the same thing when the goal of a measurement is to measure the material attribute. The use of the word "intended" in this definition is intentional and important. This is because it may be impossible to measure what one intends to measure. Further, due to experimental artifacts, what was measured during the measurement may not be what was intended [13]. These points are reminders that there are no perfect measurements and that all measurements have false positives and false negatives. This concept is key to misunderstandings surrounding potency tests.

It is important to independently define the "measurand" (the attribute being measured) and the "measurement", according to the International Vocabulary of Metrology (VIM) [12].

> *Measurement: "process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity" [12]*

Table 2 Potency tests and clinical trial designs for the 27 US-approved therapies containing live human cells

Type	Product (year)	Potency Tests (from FDA Summary Basis for Regulatory Action)	Clinical Trial Design
HSCs (allogeneic hematopoietic progenitor cell therapies)	Hemacord (2011)	*FDA SBRA:* i) Total nucleated cell number; ii) viability of CD45+ cells; iii) viable CD34+ cell count; iv) CFU	Single-arm
	Clinimmune (2012)	*FDA SBRA:* i) Total nucleated cells; ii) viability of total nucleated cells; iii) viable CD34+ cell count; iv) redacted	Single-arm
	Ducord (2012)	*FDA SBRA:* i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted; v) redacted	Single-arm
	Lifesouth (2013)	*FDA SBRA:* i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted	Single-arm
	Bloodworks (2016)	*FDA SBRA:* i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted	Single-arm
	Allocord (2016)	*FDA SBRA:* i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cell count; iv) CFU	Single-arm
	Clevecord (2016)	*FDA SBRA:* i) Total nucleated cell number; ii) viable nucleated cells; iii) viable CD34+ cell count; iv) redacted	Single-arm
	MD Anderson (2018)	*FDA SBRA:* i) Total CD34+ count; ii) total nucleated cell count; iii) nucleated red blood cell; iv) viability of nucleated cells; v) viable CD34+ cells; vi) CFU assay	Single-arm
CAR T-cells (chimeric antigen receptor T-cell therapy)	Kymriah (2017)	*FDA Panel Meeting Slides:* i) Determination of CAR expression by flow cytometry; ii) Interferon-γ production by product upon stimulation with CD19+ cells	Single-arm
	Yescarta (2017)	*FDA SBRA:* i) Cell viability; ii) anti-CD19 CAR expression; iii) redacted *Papadouli et al., 2020:* Interferon-γ production by product upon stimulation with CD19+ cells	Single-arm
	Tecartus (2020)	*FDA SBRA:* i) Cell viability; ii) anti-CD19 CAR expression; iii) redacted	Single-arm
	Breyanzi (2021)	*FDA SBRA:* i) Redacted	Single-arm with 3 dose cohorts
	Abecma (2021)	*FDA SBRA:* i) Redacted *EMA Assessment Report:* Interferon-γ production by product upon stimulation with BCMA+ cells	Single-arm with 3 dose cohorts
	Carvykti (2023)	*FDA SBRA:* i) CAR expression from viable T cells; ii) redacted	Single-arm
Other	Provenge (2010)	*FDA SBRA:* i) Number of CD54+ cells (flow cytometry); ii) increased expression of CD54 on the surface of antigen presenting cells after culture with PAP-GM-CSF (flow cytometry)	Two-arm (compared to unactivated autologous PBMCs)
	Laviv (2011)	*FDA SBRA:* i) Cell count; ii) cell viability; iii) collagen production by the cells	Two-arm (compared to placebo)
	Gintuit (2012)	*FDA SBRA:* Histology with morphological assessments: epidermal coverage, epidermal development, basal cell layer keratinocyte viability, suprabasal cell layer keratinocyte viability, dermal thickness, fibroblast density, and matrix integrity	Two-arm (compared to standard of care)
	Maci (2016)	*FDA SBRA:* i) Cell number; ii) redacted; iii) redacted *Rapko et al., 2007:* PCR measurement of aggrecan gene expression	Two-arm (compared to standard of care)
	Stratagraft (2021)	*FDA SBRA:* Redacted	Two-arm (compared to standard of care)
	Rethymic (2021)	*FDA SBRA:* Histology-based (for tissue organization, viability and retention of important cell types believed to be important for product function)	Single-arm
	Zynteglo (2022)	*FDA SBRA:* i) Vector copy number (qPCR); ii) percent LVV+ cells; iii) colony forming cells; iv) βA-T87Q-globin quantitative protein expression; v) redacted; vi) redacted	Single arm (compared to symptoms prior to treatment)
	Skysona (2022)	*FDA SBRA:* i) Vector copy number (qPCR); ii) percent LVV+ cells; iii) percent ADLP+ cells; iv) redacted; v) redacted; vi) redacted	Single arm
	Omisirge (2023)	*FDA SBRA:* CD34+ cell fold-increase	Two-arm (compared to umbilical cord blood transplantation)
	Lantidra (2023)	*FDA Advisory Committee Meeting:* i) ELISA quantification of insulin release in glucose stimulated islets; ii) islet yield assessed by dithizone stain and microscopic quantification; iii) viability assessed by SYTO 13 green/ethidium bromide staining and microscopic evaluation	Single-arm
	Casgevy (2023)	*FDA SBRA:* i) On-target editing frequency (tracking of indels by decomposition, TIDE); ii) redacted; iii) redacted.	Single-arm
	Lyfgenia (2023)	*FDA SBRA:* i) Vector copy number; ii) redacted, iii) redacted; iv) redacted; v) redacted; vi) βA-T87Q-globin quantitative protein expression.	Single-arm
	Amtagvi (2024)	*FDA SBRA:* i) redacted, ii) redacted, iii) redacted, iv) redacted, v) dose (total viable cells), vi) redacted and vii) redacted.	Single-arm

Abbreviations: CFU = colony forming unit; ELISA = enzyme linked immunosorbent assay; FDA SBRA = Food and Drug Administration Summary Basis for Regulatory Action; PAP-GM-CSF = human prostatic acid phosphatase (PAP), an antigen expressed in prostate cancer tissue, linked to human granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune cell activator; PBMC = peripheral blood mononuclear cells; SMN = survival motor neuron; PCR = polymerase chain reaction

There may be a variety of different methods for making a measurement of a material attribute and the results of the measurements may not agree. For example, two labs may try to make the same measurement but get different results. It may be "that different experiments are inadvertently being performed (*i.e.*, there are critical experimental differences that are not accounted for)" [14]. This is not a new challenge for cellular therapies but has existed more broadly for biomedical research.

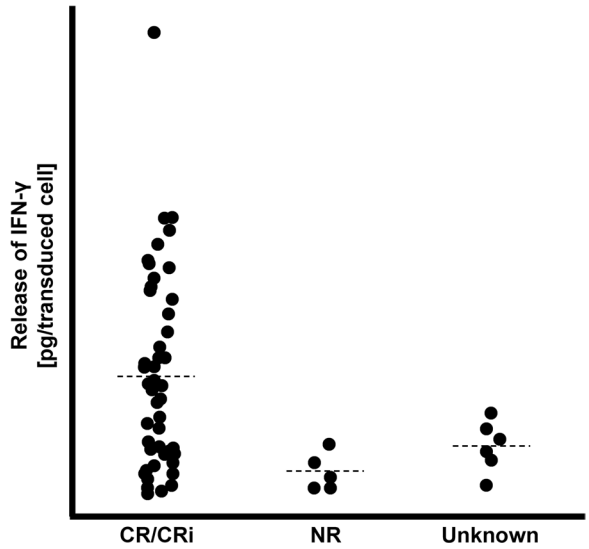

Fig. 1 Relationship between potency test results and efficacy endpoint for Kymriah clinical trial for the treatment of pediatric acute lymphoblastic leukemia after three months. The potency test was interferon-γ (IFN) production by test article upon stimulation with CD19 + cells. Data are adapted from FDA Advisory Committee Meeting materials [10]. Data are from 63 patients: 52 CR/CRi, 5 NR and 6 Unknown. CR = complete remission; CRi = complete remission with incomplete blood count recovery; NR = nonresponder; Unknown = unknown response

Mechanism of action (MOA), potency, potency test, efficacy, efficacy endpoint and efficacy endpoint test

Following are six key definitions that are charted in Fig. 2a. These definitions have been adapted from the cited sources. The source definitions are provided in Supplementary File 2 as a reference.

- *Mechanism of action (MOA):* The specific process, often pharmacologic, through which a product produces its intended effect [15, 16]
- *Potency:* The attribute of a product that enables it to achieve its intended mechanism of action [17–19]
- *Potency Test:* A test that measures the attribute of a product that enables it to achieve its intended mechanism of action [20]
- *Efficacy:* The ability of the product to have the desired effect in patients [21]
- *Efficacy Endpoint:* Attributes related to how a patient feels, functions or survives [22, 23]
- *Efficacy Endpoint Test:* A test that measures attributes related to how a patient feels, functions or survives (the authors are not aware of any existing definition for this term)

It is key that these six concepts be defined separately from one another in order to understand how they relate to one another.

Potency ≠ efficacy

A common mistake is to assume that potency and efficacy are the same thing and that a potency test that measures potency is also a measure of efficacy. This cannot be true since efficacy can only be measured by clinical response. Potency tests are laboratory assays which may or may not be a predictor of clinical response. Another way to think about it is "potency is laboratory" whereas "efficacy is clinical"; and the two are tied together by the MOA.

Potency process charts and efficacy process charts

The definitions given in Fig. 2a are applied to practical examples of two approved CTPs: a CAR T-cell therapy in Fig. 2b [10, 11, 24–32] (based on Kymriah) and tissue-engineered chondrocytes on a collagen membrane in Fig. 2c [33–37] (based on MACI). Each chart is composed of 2 triads: an effect, an attribute and a measurement which are applied to MOA in the first three boxes and to efficacy in the fourth, fifth and sixth boxes. Another way to think of these triads is 1) an effect that one is trying to achieve, 2) an attribute that can be measured to see if the effect has been achieved (the measurand), and 3) how the attributes will be measured (the measurement).

The goal of the potency and efficacy process charts in Fig. 2a is to distinguish and independently define each of the 6 components, making it easier to understand how their interrelationships can break down during product development and clinical trials.

Separate MOA from potency

A benefit of separating the "MOA" from the "potency" attribute is that it allows for the defined potency attribute to be incorrect. For example, potency of a CAR T-cell therapy could be defined as its ability to secrete IFN-γ upon recognizing target cells (Fig. 2b). However, it could be that IFN-γ secretion is not involved in the proposed MOA of the product (target cell elimination) and the product may have an unknown MOA.

Separation of "MOA" from the "potency" attribute also allows different biological activities related to the MOA to be defined as potency. For example, the potency of a CAR T-cell therapy could be defined as its ability to secrete IFN-γ upon binding to target cells (Fig. 2b), its ability to secrete IL5 upon binding to target cells or the ability of the CAR T-cells to kill target cells [31]. All, some or none of these could be solely indicative of the MOA. It may be that IFN-γ secretion is an essential factor in the MOA, and that IL5 and cell killing are not

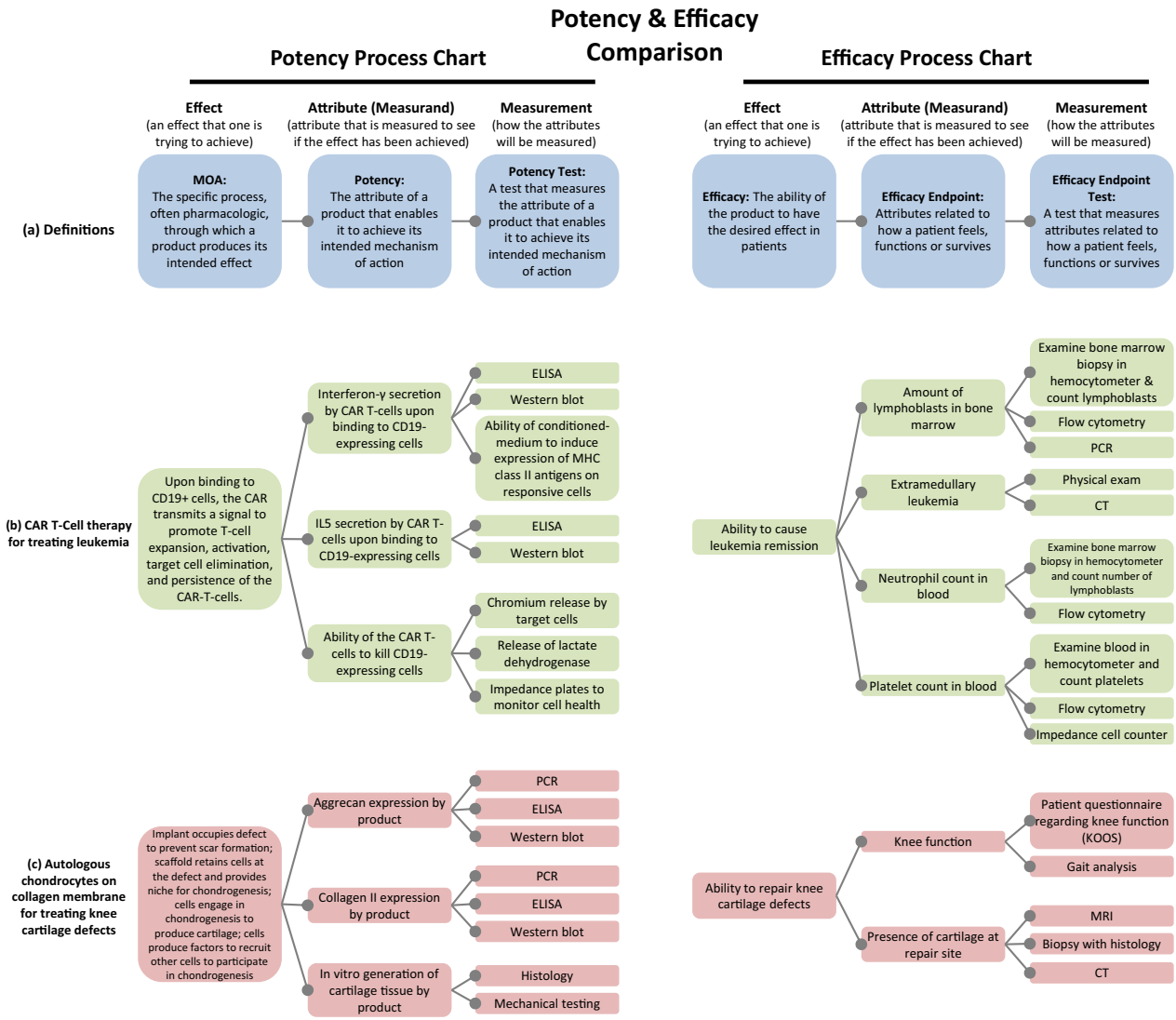

Fig. 2 Potency and efficacy process charts. Comparison of potency and efficacy definitions and processes for two example products demonstrates the parallelism between the two processes. **a** Definitions of the components of a potency and efficacy process chart. **b** Potency and efficacy process charts for treating leukemia with a CAR T-cell therapy (based on Kymriah). **c** Potency and efficacy process charts for a product for treating knee cartilage defects with chondrocytes on a collagen membrane (based on MACI). The figure graphically represents the relationships among the three inherent components of any analytical assessment: effect; attribute (measurands); and measurement; for both potency and efficacy. The examples are meant to illustrate concepts and are not intended as recommendations for potency tests or efficacy endpoint tests. The charts are not comprehensive and there may be other MOAs, attributes or tests that are not mentioned. *CT* computed tomography scan, *ELISA* enzyme linked immunosorbent assay, *IL5* interleukin 5, *KOOS* knee injury and osteoarthritis outcome score, *MHC* major histocompatibility, *MRI* magnetic resonance imaging, *PCR* polymerase chain reaction. The cited sources were used to assemble the content for (**b**) Kymriah [10, 11, 24–32] and (**c**) MACI [33–37]

major contributors. Or, maybe none of these activities are part of the MOA.

Separate potency from potency test

Likewise, "potency" and "potency test" should be distinct in the event that 1) the "potency test" is not a reliable measurement of the potency attribute and 2) there are multiple ways to measure the potency attribute. For case 1), measurement of IFN-γ secretion by CAR T-cells by enzyme linked immunosorbent assay (ELISA) (Fig. 2b) could lead to artifactual data that do not accurately measure the amount of IFN-γ released. For case 2, IFN-γ release could be measured by ELISA or Western blot and the results may not agree. These possibilities are obscured if the potency attribute is defined in terms of a specific measurement, emphasizing the value in keeping

potency (attribute or measurand) and the potency test (measurement) distinct.

Separate efficacy from efficacy endpoint

The concept of "efficacy" should remain generalized and distinct from the "efficacy endpoint", while the "efficacy endpoint" should be specific to a patient symptom or attribute. For instance, when developing a CAR T-cell therapy, developers could define "efficacy" as "ability to cause leukemia remission", which is a general definition that does not mention patient attributes or symptoms (i.e., the "efficacy endpoints") (Fig. 2b). If efficacy is defined as "amount of lymphoblasts" (instead of as the "ability to cause leukemia remission"), then developers are led to believe that "amount of lymphoblasts" is certain to be indicative of the disease status of a patient, and the possibility that "amount of lymphoblasts" is not linked to the disease state is more easily overlooked. The measurement of the "amount of lymphoblasts" in bone marrow may not be indicative of a particular blood cancer, and it may be that other efficacy endpoints should be considered, such as presence of extramedullary tumors. Having separate definitions for "efficacy" (the effect you are trying to achieve) and "efficacy endpoints" (patient attributes or symptoms) allows for the possibility that a given "efficacy endpoint" is not indicative of the disease state. This distinction is subtle but it avoids the false assumptions that lead to confusion when unexpected clinical results are observed.

Separate "efficacy endpoint" from "efficacy endpoint test"

Separation of the "efficacy endpoint" from the "efficacy endpoint test" (the measurement) makes it easier to deal with false measurement results. The VIM provides separate definitions for measurand and measurement [12], so it makes sense to do the same here. For example, the amount of lymphoblasts in bone marrow may be used as an efficacy endpoint for leukemia treatment (Fig. 2b). The measurement of the amount of lymphoblasts could be conducted by hemocytometer counting (efficacy endpoint test) but the results could be deceptive. There may be an interference in the hemocytometer count (e.g., cell clumping, debris) causing it to give false readings. The efficacy endpoint should be defined without referring to a specific measurement method, so that the possibility of false results is not obscured.

Separation of the "efficacy endpoint" from "efficacy endpoint test" makes it easier to deal with different measurements of an attribute. The number of lymphoblasts in marrow could be measured by counting in a hemocytometer, by PCR (polymerase chain reaction) or by flow cytometry; and the three measurements may not agree [30]. If the efficacy endpoint is defined as a hemocytometer measurement of lymphoblasts, then the circumstance of multiple measurements that may not agree becomes intractable.

"Potent but not efficacious" and "not potent but efficacious"

Since potency and efficacy cannot be the same thing (because potency is measured by a lab test and efficacy is measured by clinical trial), then it follows that a therapeutic could be "potent but not efficacious" or "not potent but efficacious." These are confusing scenarios, but it is important to consider them, since they get to the heart of the challenge surrounding MOA, potency and efficacy.

Potent but not efficacious: wrong patient population

An example of "potent but not efficacious" is giving a potent chemotherapeutic to a patient with bronchitis. Imagine that the product is truly potent for its intended MOA and is truly efficacious when used as intended in the treatment of cancer. Yet, the chemotherapeutic will not help the patient with bronchitis. This could be a case of a misdiagnosis or treating the wrong patient population.

This example could seem irrelevant, but identifying the appropriate indication and selecting the appropriate patient inclusion and exclusion criteria for a clinical trial are critical. When statistical significance is not observed in a costly and lengthy clinical trial, there are often controversial post-hoc subgroup analyses that identify responsive cohorts. A better understanding of MOA helps with identification of patients most likely to respond prior to starting a clinical trial. An example is companion diagnostics [38], such as the well-known example of breast cancer screening to treating HER2/*neu*-positive patients with Herceptin, an anti-HER2/*neu* antibody drug [39]. The FDA website currently lists 169 companion diagnostics, which highlights the value of identifying the correct patient population [38].

Another example is the indication for Kymriah, which is carefully worded with 5 qualifiers to specifically identify those patients most likely to benefit from treatment: "patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse" [28] (Additional file 1). Another example is the carefully worded indication for Lantidra which has six qualifiers: "The treatment of adults with Type 1 diabetes who are unable to approach target HbA1c because of current repeated episodes of severe hypoglycemia despite intensive diabetes management and education." Careful thinking and strategy are required to write

CTP indications so that patients for whom the product is most likely to be efficacious are identified.

Potent but not efficacious: incorrect hypothesis regarding the disease mechanism

Another example of "potent but not efficacious" is having an incorrect hypothesis regarding the MOA for the targeted indication. Imagine the therapeutic is truly potent and achieves its intended MOA. Yet, it does not benefit the patient population because the hypothesis regarding the MOA is wrong for the intended indication. Achieving the intended MOA does not have an effect in treating the intended indication. This exercise highlights the importance of separating the concepts of potency and efficacy. If potency and efficacy are equated to one another, then "potent but not efficacious" becomes inconceivable.

Another useful example for understanding "potent but not efficacious" is as follows. If a person takes an aspirin for a headache and the headache is not alleviated, does that mean the aspirin is not potent? The aspirin is probably still potent, inhibits cyclooxygenase and blocks prostaglandin synthesis to achieve its intended MOA [40]. Yet, the aspirin is not efficacious in curing the headache. The headache may be caused by factors unrelated to cyclooxygenase or prostaglandins.

Not potent but efficacious: alternate MOA

An example of "not potent but efficacious" could be a therapeutic that is effective due to an MOA that is not the proposed MOA. Imagine the therapeutic is truly *not potent* for its intended MOA, but the therapeutic is truly effective in treating the intended indication. It could be that the proposed MOA was incorrect, and the product is effective due to an alternate MOA (perhaps an unknown biological activity). To revisit the CAR T-cell example discussed in Fig. 2b, potency of a CAR T-cell therapy may not be due to its ability to secrete IFN-γ upon binding to target cells, but instead could be due to secretion of IL5 upon binding to target cells or the ability of the CAR T-cells to kill target cells via perforin-granzyme or Fas–Fas ligand interactions [41].

Not potent but efficacious: false negative potency test

Another example of "not potent but efficacious" is a false negative potency test result. Imagine that a therapeutic is truly potent. It achieves its intended MOA and achievement of the intended MOA is truly effective in treating the intended indication. Yet, the potency test indicates that the therapeutic is not potent. In this scenario, the potency test may be a poor measure of true potency and may yield incorrect results: that the therapeutic is not potent. In this case, the potency test is giving false negative results. For example, the potency test may not be stable and reagent degradation results in intermittent false negatives.

MOA case studies: aspirin and acetaminophen

Aspirin MOA

The MOA of aspirin (acetylsalicylic acid) is useful to consider. Aspirin is considered the most widely used drug of all time [42]. For thousands of years, plant extracts, such as from willow bark which contains salicylate, have been used to treat rheumatism (joint inflammation) and pain. In 1897, Bayer reported a synthetic version of acetylsalicylic acid that was named aspirin. In the early 1970s, research by John Vane shed light on aspirin's MOA with the discovery of cyclooxygenase (COX-1) and its acetylation by aspirin [43–45], leading to the 1982 Nobel Prize in Medicine [46]. However, other COX isoforms were discovered; COX-2 in 1991 [47] and COX-3 in 2002 [48]; and COX-3 was inhibited by aspirin. In the 2000s, additional MOAs for aspirin were elucidated: i) uncoupling of oxidative phosphorylation [49], ii) promotion of nitric oxide synthesis to inhibit inflammation [50] and iii) inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation to inhibit inflammation [51]. This evidence suggests that aspirin has multiple (and possibly multifactorial) MOAs and can affect at least 5 pathways: COX-1, COX-3, oxidative phosphorylation, nitric oxide and NF-κB. Future studies may identify new MOAs for aspirin. Thus, even after thousands of years of use, and being the most widely used drug of all time, the MOA of aspirin is still unclear.

Acetaminophen MOA

Another example of a widely used drug whose MOA is uncertain is acetaminophen (paracetamol). Acetaminophen, a small molecule drug with a molecular mass of 151 g/mol, is probably the most widely prescribed drug for children and is used for pain management and fever reduction. It was first synthesized in 1878, first used clinically in 1887, and was first marketed in the US in the 1950s [52]. Acetaminophen's package insert states that "The precise mechanism of the analgesic and antipyretic properties of acetaminophen is not established but is thought to primarily involve central actions" [53]. There is evidence that acetaminophen inhibits prostaglandin synthesis [54], inhibits COX-3 [48], activates a vanilloid receptor (TRPV1) [55] and modulates the cannabinoid system [56].

CTPs vs. "aspirin and acetaminophen"

Aspirin and acetaminophen, small molecule drugs with molecular masses of 180 g/mol and 151 g/mol, respectively, each has multiple activities. In small molecule drug discovery, the term polypharmacology means "the

binding of a drug to multiple target proteins, with clinical effects being mediated through the modulation of the set of protein targets" [57]. The precise MOA for many drugs is unknown, which indicates that knowing the MOA is desirable but unnecessary for having an effective therapeutic [58]. A cell therapy delivers millions of metabolically active cells to a patient, where each cell is a bag of thousands of different molecules, including proteins, lipids, carbohydrates and nucleic acids, and each of these molecules could have multiple activities. Given the fact that the MOAs for aspirin and acetaminophen are still being investigated, determining the precise MOA for a cell-containing therapeutic seems especially challenging. Further, without a good understanding of the MOA, it is hard to imagine that there will be good potency tests, given that potency tests should be based on the MOA. Given the tools currently at our disposal, it may be useful to consider the establishment of a product's MOA as an aspirational goal, instead of as something that can be known with certainty. This may change with development of improved technology for determining MOA.

Clinical trials are not designed to establish MOA

Example application: establishing MOA for a CAR T-cell product

It is useful to consider an example application for establishing the MOA of a CAR T-cell therapy directed against leukemia cells expressing a target surface antigen (based on Kymriah). The proposed MOA for this product is that upon binding to CD19 + cells (cancer cells), the CAR transmits a signal to promote T-cell expansion, activation, target cell elimination, and persistence of the CAR-T-cells.

Table 3 shows experiments that can be used to establish the MOA. The tests can be run in cell culture using an in vitro cell killing assay, in animals using an animal model for leukemia and in a human clinical trial to treat leukemia patients. The test article is "CAR T-cells directed against leukemia cells expressing a target surface antigen" as shown in the first row of the table. The second row is a negative control, to assess if the test article has an effect above background signal. The negative control could be an untreated well for cell culture, could be a sham CAR T-cell delivery for the animal (saline injection) and may not be possible for humans since it may not be ethical to leave humans untreated. The third row is "Standard of care," which is not applicable for cell culture, but could be an existing chemotherapy for animals and humans. The fourth row is "Unmodified T-cells" only, which can establish if CAR expression is required for the MOA. The fifth row is "CAR T-cells with empty CAR," which can establish if a specific CAR must be expressed or if any CAR can suffice. The sixth row is "Dead CAR T-cells" which tests if live cells are required. Finally, the seventh row is "Fibroblasts expressing CAR" to determine if T-cells are required or if any cell can suffice. Note that most experiments in Table 3 can be done in cells or animal models, but, due to ethical considerations, only one or two of the experiments can be done in CTP clinical trials. This example application highlights the difficulty in establishing MOA in humans. Clinical trials are designed to assess efficacy and safety, not to establish MOA or validate a potency test.

Example application: establishing MOA for a tissue engineered medical product

Another example application is given in Table 4: establishing the MOA of a tissue engineered medical product composed of autologous cultured chondrocytes on a collagen membrane for treating knee cartilage defects (based on MACI). The proposed MOA is cartilage regeneration by the following activities: i) the implant will occupy the trauma site to prevent scar formation, ii) the cells will proliferate and secrete cartilage matrix that will form new cartilage tissue, iii) the scaffold will help retain the cells at implantation site providing a supportive niche for the cells to proliferate, differentiate and make new cartilage and iv) the cells will secrete factors to recruit other cells to the implantation site to support cartilage regeneration.

The test article is "Cells + scaffold" as shown in the first row of Table 4. The second row is "Negative control," to assess if the test article has an effect above background levels. The negative control could be an untreated well for cell culture, a sham surgery for animals and may not be applicable for humans (since it may not be ethical to leave humans untreated). The third row is "Standard of care," which is not applicable for cell culture, but could be microfracture for animals and humans. The fourth row is "Scaffold" only, which can establish if cells are required for the MOA. The fifth row is "Cells" only, which can establish if the cells by themselves can regenerate cartilage or if the scaffold is required. The sixth and seventh rows use dead cells instead of live cells, to establish that live cells are required for cartilage regeneration. The eighth and ninth rows use non-relevant cells, such as fibroblasts, to establish that the chondrocytes are required for the MOA. The tenth and eleventh rows use scaffolds with the wrong porosity (out of specification), to establish that scaffolds with the specified porosity are required for the MOA. All the experiments could be conducted in cells or animals, but only one, maybe two, can be conducted in humans. These examples demonstrate how clinical trials are not designed to establish the product's MOA.

Table 3 Example application for establishing MOA for a hypothetical product: chimeric antigen receptor (CAR) T-cell therapy directed against leukemia cells expressing a target surface antigen (based on Kymriah)

Treatment	What does it test?	Cell Culture: Assess CAR T-cell killing of cells expressing the target surface antigen	Animal Model: Treat animals with leukemia	Human Clinical Trial: Treat leukemia patients & assess remission (by lymphoblasts in bone marrow, blasts in peripheral blood, extramedullary disease, neutrophils, platelets)
1) CAR T-cells directed against leukemia cells expressing a target surface antigen	This is the test article	✓	✓	✓
2) Negative control (placebo, sham)	Does test article have an effect greater than natural healing response?	✓ (untreated dish of cells expressing the target surface antigen)	✓ (sham infusion)	*May not be ethical*
3) Standard of care (chemotherapy)	How does test article compare with current treatment options?	*not applicable*	*May not be an existing treatment*	*May not be an existing treatment*
4) Unmodified T cells	Is the CAR necessary? (Can T-cells work alone?)	✓	✓	X
5) CAR T-cells with empty CAR (CAR lacks binding site for leukemia cell receptor)	Is a specific CAR required or can any CAR suffice? (can CAR work without being directed towards leukemia cell surface antigen?)	✓	✓	X
6) Dead CAR T-cells directed against leukemia cells expressing a target surface antigen	Are live cells required? (Can dead cells suffice?)	✓	✓	X
7) Fibroblasts expressing CAR directed against leukemia cells expressing a target surface antigen	Are T-cells required? (Will other cell types work?)	✓	✓	X

**The first column in each row indicates control experiments that could be performed to establish the product's MOA. Grey shading in the first row indicates the test article, which is the product in its intended form. The second column of each row indicates what each experiment would test. Three different test systems are given in the third column (cell culture), fourth column (animal model) and fifth column (human clinical trial). Green check marks indicate that an experiment can be performed in the given test system while red X indicates that it is not appropriate to conduct the experiment in the test system. All clinical trials for the 27 US FDA approved CTPs conducted to date are either single-arm or two-arm trials. The experiments described in the first column are meant to serve as examples and are not meant to serve as recommendations for experiments that should be conducted to establish the MOA of a CAR T-cell therapy.*

Table 4 Example application for establishing MOA for a hypothetical product: Autologous cultured chondrocytes on collagen membrane for treating knee cartilage defects (based on MACI)

Treatment	What does it test?	Cell Culture: Assess cartilage formation in cell culture dish using histology	Animal Model: Implant in animal with cartilage defect & assess regeneration by histology	Human Clinical Trial: Implant in patients with knee cartilage defect & assess cartilage repair using a questionnaire (KOOS)
1) Cells + scaffold	This is the test article	✓	✓	✓
2) Negative Control (placebo, sham)	Does test article have an effect greater than natural healing response?	✓ (dish with medium only)	✓ (sham surgery)	*not applicable*
3) Standard of care (microfracture)	How does test article compare with current treatment options?	*not applicable*	✓	✓
4) Scaffold	Can scaffold work alone? (are cells required?)	✓	✓	X
5) Cells	Can cells work alone? (is scaffold required?)	✓	✓	X
6) Dead cells	Are live cells required? (can dead cells suffice?)	✓	✓	X
7) Dead cells + scaffold		✓	✓	X
8) Non-relevant cells (fibroblasts)	Are chondrocytes required? (can any cell suffice?)	✓	✓	X
9) Non-relevant cells (fibroblasts) + scaffold		✓	✓	X
10) Scaffold w/wrong porosity	Are scaffold specs meaningful? (can out-of-spec scaffolds suffice?)	✓	✓	X
11) Cells + Scaffold w/wrong porosity		✓	✓	X

**The first column in each row indicates control experiments that could be performed to establish the product's MOA. Grey shading in the first row indicates the test article, which is the product in its intended form. The second column of each row indicates what each experiment would test. Three different test systems are given in the third column (cell culture), fourth column (animal model) and fifth column (human clinical trial). Green check marks indicate that an experiment can be performed in the given test system while red X indicates that it is not appropriate to conduct the experiment in the test system. All clinical trials for the 27 US FDA approved CTPs conducted to date are either single-arm or two-arm trials. The experiments described in the first column are meant to serve as examples and are not meant to serve as recommendations for experiments that should be conducted to establish the MOA of a cartilage tissue therapy. (KOOS = Knee injury and Osteoarthritis Outcome Score)*

Clinical trial structures for 27 CTPs approved in US

Table 2 (fourth column) shows the clinical trial structures (i.e., designs) for the 27 CTPs approved in the US. Only two of these 27 trials provide relevant insight on the MOA. Twenty one of the 27 trials (78%) were single-arm which cannot substantively inform the MOA. Six trials (22%) were two-arm, of which four did not shed much light on MOA: one compared to placebo (Laviv) and three were compared to standard of care (Gintuit, MACI, Stratagraft). One-arm trials, as well as two-arm trials that compare to placebo, untreated or standard of care, are useful for assessing efficacy, but shed little light on MOA. Thus, 25 of the 27 CTP trials (93%) did not substantively inform the MOA.

However, two of the 27 trials (7%) shed light on the MOA. The Provenge trial compared the test article, activated peripheral blood mononuclear cells (PBMCs), to unactivated PBMCs. This tested whether activated PBMCs were required for efficacy. Omisirge, umbilical cord blood (UCB) cells expanded ex vivo in the presence of nicotinamide, was compared to standard of care, which was untreated UCB cells. This tested whether cells expanded in the presence of nicotinamide performed comparably to unmanipulated UCB cells. Thus, only two of the clinical trials for the 27 approved CTPs had any real bearing on the MOA in humans.

Control experiments in a research paper

To take the examples further, consider the challenge of achieving the evidence in a CTP clinical trial that would be required for a peer-reviewed basic research publication. Imagine if a research paper were submitted that reported only one experiment, the "cells + scaffold" experiment from Table 4, to assess cartilage regeneration in an animal model. Furthermore, imagine for this hypothetical study that no controls were run: no negative control, no cells only, no scaffold only, and no microfracture. The manuscript would likely be rejected with the reviewers requiring that the control experiments be conducted. Applying this same standard is neither feasible nor ethically responsible for CTP clinical trials, further illustrating the difficulty in establishing MOA in clinical trials.

CTP clinical trials are designed to assess efficacy—not establish MOA

Given that only single-arm or two-arm clinical trials are conducted for CTPs, it may be unrealistic at this time to think that the MOA of a CTP could be established in humans with certainty. Indeed, CTP clinical trials are not designed to establish MOA but are instead designed to assess safety and efficacy. Even for cells and animals, where many types of experiments can be conducted (Tables 3 and 4), establishing the MOA is largely aspirational, as discussed above in the aspirin example. Aspirin affects at least five biochemical pathways which leads to uncertainty regarding its efficacy-relevant MOA in cells or animals.

Considerations for the development of potency tests

Potency test validation

Careful validation procedures for characterizing potency test performance are critical for assay reliability. Potency tests should be validated for specificity, sensitivity, linearity, range, accuracy, precision, repeatability (within lab variability), reproducibility (between lab variability), detection limit, quantification limit, robustness and fit for purpose [18, 59–61]. It should be established that the potency test can perform within defined specifications to help assure consistency in the manufactured product. Those specifications are set by measuring reference samples under a variety of conditions, modifying the assay to reduce uncertainty, and qualifying the assay response with respect to its precision and accuracy. The resulting criteria that are established for precision and accuracy in measurement of the reference sample are specifications that must be met when running the assay with the reference sample in parallel with the CTP sample.

Matrix approach to potency tests

Use of several different potency tests, or a potency assay matrix [62], may be useful for CTPs for several reasons. First, CTPs likely have multiple MOAs. Second, there may be multiple product attributes that could be used as a potency attribute for any one of the MOAs for a given product. As discussed for a CAR T-cell therapy, its potency attribute could be defined as its ability to secrete IFN-γ upon binding to target cells (Fig. 2b), its ability to secrete IL5 upon binding to target cells, the ability of the CAR T-cells to kill target cells or a complex response that involves all three activities. Third, there may be a variety of ways to measure a given potency attribute. As discussed above, CAR T-cell release of IFN-γ release could be measured by ELISA or Western blot.

An assay matrix is also useful for the application of orthogonal methods for measuring a potency attribute in order to improve measurement confidence. Orthogonal measurements use different physical principles to measure the same property of the same sample with the goal of detecting method-specific biases and interferences [63]. The comparison of orthogonal methods can establish confidence in the accuracy of an assay. An example could be measurement of the ability of CAR T-cells to kill CD19-expressing cells by chromium release of target cells and by impedance plates (Fig. 2c). These measurements are orthogonal to one another because they are

based on different physical principles: chromium release monitors cell health by assessing membrane integrity while impedance plates monitor cell health by the cells' ability to adhere to the plate (since impedance decreases when cells detach). When a similar measurement result is obtained from the two techniques, confidence in the results is enhanced.

Potency test variability, product variability, efficacy endpoint test variability and patient variability

It is difficult to determine if variability in potency test results is due to variability inherent in the test measurement, in the product or both. There may be variability in the biological reagents used for the potency test, such as the target cells in an IFN-γ release assay or in a cell-killing assay. There is well-known variability in antibody-based detection, such as detection of a secreted protein, like IFN-γ, by an ELISA. Assay validation strives to control for these variables with additional measurements that establish criteria for reagent storage and handling. There may be variability in the CTP, both within a batch or between batches.

Another challenge is variability in the patient population receiving the treatment. Patients in a population may vary from one another due to inherent biological, environmental and clinical factors, including previous treatments. Each patient's condition may require different functionalities (*i.e.,* MOAs) from the product. Finally, there is variability in the efficacy endpoint testing, such as determining the amount of lymphoblasts in bone marrow [30] for a CAR T-cell clinical trial, that make it challenging to assess efficacy. Reducing variability in the product itself, the product manufacturing process, product testing (e.g., potency test), and efficacy endpoint tests, makes it more likely that a statistically significant clinical benefit to patients can be detected in a clinical trial. Reducing patient variability, by narrowing the indication, or refining the inclusion and exclusion criteria, may also increase the likelihood of detecting efficacy in a clinical trial.

Short product shelf life

Short product shelf life may limit the availability of potency test results at the point of product release if the product has a shelf life that is shorter than the time it takes to conduct the potency test. These cases require rapid alternative methods of obtaining equivalent data for release. If a compendial method is required and that assay exceeds the shelf-life, a two-stage release may be appropriate using an alternative rapid assay for initial release followed by confirmation of the batch disposition by a validated, longer-term test.

Stability and manufacturing changes

A potency test ought to be sensitive to product stability [7], such that products that have degraded from aging should demonstrate low potency test results. When changes are made to a manufacturing process, potency tests are important for assessing comparability between newer and older lots to assess how the changes may affect product performance [64].

Potency assurance strategy

FDA recently released a new draft guidance for a potency assurance strategy for cell and gene therapy products [8]. "A potency assurance strategy is a multifaceted approach that reduces risks to the potency of a product through manufacturing process design, manufacturing process control, material control, in-process testing and potency lot release assays." The new guidance emphasizes a lifecycle approach to potency that is grounded in quality risk management, where potency tests are considered throughout the product lifecycle from product development to licensure [65]. A lifecycle approach could allow the potency tests to change during product development as knowledge of the MOA, potency tests and risks to product potency is gained.

Standards for potency tests

An ASTM "Standard Guide for Cell Potency Assays for Cell Therapy and Tissue Engineered Products" was published in 2019 that summarized current perspectives on potency from FDA Guidance Documents, US Pharmacopeia (USP), International Conference on Harmonization (ICH) and European Medicines Agency (EMA) [61]. A feasibility study on potency published in 2022 found that potency tests may not yet be ready for standards development [5]. However, some assays may be ready for development into standard test methods [66], such as CAR T-cell potency tests, cell viability tests and methods for quantifying the fraction of viable stems cells in a cell preparation. Of the six CAR T-cell therapies on the market, three of them reveal IFN-γ secretion and CAR expression as potency tests. In addition, 16 of the 27 approved cell therapies (59%) cite cell viability tests as a potency test (Table 2). These numbers may be higher, since many potency tests are redacted. The use of a cell viability test as a CTP potency test may not be ideal, since cell viability tests may not be specific enough with regard to the MOA. There are many CAR T-cell therapies and other types of cell therapies under development that might benefit from standard test methods for IFN-γ secretion, CAR expression and cell viability. Finally, methods for quantifying the specific fraction of viable tissue stem cells in cell preparations are under

development as standard test methods for assessing the potency of stem cell therapy products [67]. Standard test methods should be vetted by interlaboratory studies to identify sources of variability and to assess reproducibility when the tests are conducted in different labs, by different operators and with different equipment [68].

The future of MOA and potency tests

New methods for determining the MOA of CTPs may be developed in the future. The biological activity assessed in potency tests may be the result of complex interactions. Multi-omics is a promising approach where many omics modalities are used to collect data from the product and patients such as genomics, transcriptomics, proteomics, secretomics, metabolomics and lipidomics [69–71]. Design of experiments and multifactorial experimental designs hold promise for determining how complex biological processes operate [72, 73]. Machine learning and artificial intelligence may be useful for determining MOA and potency attributes from omics data. Systems level thinking, such as computational systems modeling, which takes a top-down approach that focuses on the macroscopic behavior of complex systems to predict behavior, may be useful for predicting the non-linear and emergent behavior of biological systems [74]. Automation and robotics for accelerated and higher throughput testing may also be important [75]. Collection of large amounts of product data and patient data during clinical trials will be helpful, so that the relationship between product attributes and patient attributes can be used to improve understanding of MOAs. It would be helpful if some of these efforts occur in the public domain, so that the data are publicly available [6] (Fig. 1). This may require industrial consortia due to the high costs and amount of effort that are required for clinical studies. Public domain product and patient data would allow the data science community at large to participate in developing innovative analytical methods for determining MOAs.

Potency and MOA are useful concepts

Despite the challenges associated with MOA, potency, and efficacy that are discussed herein, potency tests and efforts to establish an MOA are important for CTPs. Human CTP clinical trials should have a rational and scientific underpinning where a sensible MOA is used as the motivation for product development and human investigation. Having an MOA and potency test that can withstand scientific scrutiny assures that human testing is conducted with a sound basis in scientific reasoning. Biologics are used for their biological activity and it makes sense to assess the quality of a CTP using a potency test that assesses its biological activity. This perspective offers a framework for interpreting what has been experienced for the US-approved CTPs. This perspective does not suggest that clinical trials should have multiple arms that include more of the experiments shown in Tables 3 and 4. The limitations of clinical trials may feel restrictive from a scientific standpoint, but these constraints are necessary to protect human subjects. The field of CTPs is rapidly evolving and the responses to the challenges presented herein will undoubtedly need to evolve as well.

Conclusions

There are several key takeaways from this perspective:

- A measurand is "the quantity or property *intended* to be measured", reminding us that all measurements have false positives and false negatives.
- A product attribute is often a measurand and should be defined independently from the measurement of the attribute.
- Potency ≠ efficacy (ideally, potency test results correlate with efficacy endpoint test results)
- A CTP can be "potent but not efficacious" or "not potent but efficacious." The CTP development goal is to achieve "potent and efficacious."
- Clinical trials are not designed to establish an MOA or validate a potency test; instead, clinical trials are designed to assess efficacy and safety.
- The clinical trials for the 27 US-approved CTPs were one- or two-arm trials; it is challenging to establish the MOA in humans or validate a potency test with any level of certainty with only one or two arms.
- Potency tests and efforts to establish an MOA are essential, since clinical trials should have a rational and scientific basis in a plausible MOA that guides product development and human investigation.

MOA, potency, potency test, efficacy, efficacy endpoint and efficacy endpoint tests should be independently defined to improve clarity during discussions concerning correlations between potency test results and clinical outcomes. Clarification of these independent terms will help to avoid hidden assumptions that result when concepts such as potency and efficacy are conflated. The ideas and observations presented herein may be helpful to product developers for setting realistic goals for understanding a product's MOA and for establishing correlations between potency tests and clinical efficacy.

Abbreviations

ALL	Acute lymphoblastic leukemia
CTP	Cell therapy product
CAR	Chimeric antigen receptor
COX	Cyclooxygenase

ELISA	Enzyme linked immunosorbent assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
IFN-γ	Interferon-γ
ICH	International Conference on Harmonization
MOA	Mechanism of action
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PAP-GM-CSF	Human prostatic acid phosphatase (PAP) linked to human granulocyte–macrophage colony-stimulating factor
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
SBRA	Summary Basis for Regulatory Action
US	United States
USP	US Pharmacopeia
VIM	Vocabulary of Metrology

Supplementary Information

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Additional file 1. Key aspects for each of the 27 US-approved cell therapy products are summarized including product name, year approved, sponsor, product description, indication, clinical trial structure, efficacy endpoints, mechanism of action, potency test, comments and references.

Additional file 2. Key definitions that are referred to in the main text.

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Supplementary File 1

***Simon et al., Mechanism of Action, Potency and Efficacy: Considerations for Cell* Therapies**

Cell Therapy Products Approved by FDA for Marketing in the USA

Summary: The following pages contain a 1-page summary of each of the 27 cell therapy products (CTPs) approved for marketing in the US. Key aspects of each product are summarized including product name, year approved, sponsor, product description, indication, clinical trial structure, efficacy endpoints, mechanism of action, potency test, comments and references. Two cell-containing products are omitted from this list, Apligraf (approved 1998) and Dermagraf (approved 2001), because they were reviewed by the US FDA as devices and potency tests were not required. A key resource is an FDA webpage that provides regulatory documentation for the 27 approved CTPs: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products.

Product: Kymriah (tisagenlecleucel) **Year approved:** 2017

Sponsor: Novartis Pharmaceuticals

Description: "KYMRIAH™ (tisagenlecleucel) is a CD19-directed genetically modified autologous T cell immunotherapy comprised of autologous T cells that are genetically modified using a lentiviral vector to encode an anti-CD19 chimeric antigen receptor (CAR). The CAR is comprised of a murine single-chain antibody fragment (scFv) specific for CD19, followed by a CD8 hinge and transmembrane region that is fused to the intracellular signaling domains for 4-1BB (CD137) and CD3 zeta. KYMRIAH is prepared from the patient's peripheral blood mononuclear cells, which are obtained via a standard leukapheresis procedure. The mononuclear cells are enriched for T cells, then transduced with the lentiviral vector containing the anti-CD19 CAR transgene, and activated with anti-CD3/CD28 antibody coated beads. The transduced T cells are expanded in cell culture, washed, and formulated into a suspension, which then is cryopreserved. The product must pass a sterility test before release for shipping as a frozen suspension in a patient-specific infusion bag(s). The product is thawed prior to administration [see Dosage and Administration (2.3), How Supplied/Storage and Handling (16)]. The thawed product is a colorless to slightly yellow suspension of cells."

Indication: Indicated for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse.

Clinical Trial Structure: Single arm; compared to historical data for standard of care

Efficacy Endpoints: Overall remission rate (complete remission defined as <5% lymphoblasts in bone marrow by morphology, circulating blasts <1% in peripheral blood, no evidence of extramedullary disease, neutrophils >1.0×10^9/L, platelets >100×109/L, and no platelet and/or neutrophil transfusions within 7 days of peripheral blood sample for disease assessment)

MOA (Package Insert): "KYMRIAH is a CD19-directed genetically modified autologous T-cell immunotherapy which involves reprogramming a patient's own T-cells with a transgene encoding a chimeric antigen receptor (CAR) to identify and eliminate CD19-expressing malignant and normal cells. The CAR is comprised of a murine single-chain antibody fragment which recognizes CD19 and is fused to intracellular signaling domains from 4-1BB (CD137) and CD3 zeta. The CD3 zeta component is critical for initiating T-cell activation and antitumor activity, while 4-1BB enhances the expansion and persistence of KYMRIAH. Upon binding to CD19-expressing cells, the CAR transmits a signal to promote T-cell expansion, activation, target cell elimination, and persistence of the KYMRIAH cells."

Potency Testing (FDA Panel Meeting Slides): i) Determination of CAR expression by flow cytometry; ii) Interferon-γ production by product upon stimulation with CD19+ cells

Potency Comments (FDA Briefing Document): "In the clinical trials, IFN-γ production varied greatly from lot-to-lot, making it difficult to correlate IFN-γ production in vitro to tisagenlecleucel safety or efficacy."

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Product: Yescarta (axicabtagene ciloleucel) **Year approved:** 2017

Sponsor: Kite Pharma

Description: Genetically modified, antigen-specific autologous T-cells reprogrammed to target cells that express CD19

Indication: For the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Complete remission (as defined complete disappearance of all detectable clinical evidence of disease and symptoms; typically assessed by absence of masses by positron emission tomography (PET), computed tomography (CT) to assess absence of liver/spleen enlargement, bone marrow biopsy)

MOA (package insert): "YESCARTA, a CD19-directed genetically modified autologous T-cell immunotherapy, binds to CD19-expressing cancer cells and normal B cells. Studies demonstrated that following anti-CD19 CAR T-cell engagement with CD19-expressing target cells, the CD28 and CD3-zeta co-stimulatory domains activate downstream signaling cascades that lead to T-cell activation, proliferation, acquisition of effector functions and secretion of inflammatory cytokines and chemokines. This sequence of events leads to killing of CD19-expressing cells."

Potency Testing:

- *FDA SBRA*: i) Cell viability; ii) anti-CD19 CAR expression; iii) redacted
- *Papadouli et al., 2020:* Interferon-γ production by product upon stimulation with CD19+ cells

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Product: Tecartus (brexucabtagene autoleucel) **Year approved:** 2020

Sponsor: Kite Pharma

Description: TECARTUS is comprised of genetically modified, antigen-specific autologous T-cells reprogrammed to target cells that express CD19, an antigen expressed on the surface of healthy and malignant B cells. The TECARTUS chimeric antigen receptor (CAR) protein has a murine single chain variable fragment (scFv) specific to human CD19 linked to two signaling domains derived from human CD28 and CD3ζ. The CAR protein plays a critical role in TECARTUS function, mediating T-cell activation and anti-tumor effector function following binding of the scFv to CD19. The CAR expressed in TECARTUS is identical to that in YESCARTA (axicabtagene ciloleucel), a CD19-directed genetically modified autologous T-cell immunotherapy approved in 2017 for relapsed or refractory large B cell lymphoma. TECARTUS differs from YESCARTA in that T-cells are enriched during the TECARTUS manufacturing process; T-cell enrichment is not performed during YESCARTA manufacture.

Indication: Relapsed/refractory mantle cell lymphoma

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoint: Objective response rate [typically assessed by positron emission tomography-computed tomography (PET-CT) for presence of lesions, bone marrow biopsy, lumbar puncture for examination of cerebral spinal fluid (CSF)]

MOA (package insert): "TECARTUS, a CD19-directed genetically modified autologous T-cell immunotherapy, binds to CD19-expressing cancer cells and normal B cells. Studies demonstrated that following anti-CD19 CAR T-cell engagement with CD19-expressing target cells, the CD28 and CD3-zeta co-stimulatory domains activate downstream signaling cascades that lead to T-cell activation, proliferation, acquisition of effector functions, and secretion of inflammatory cytokines and chemokines. This sequence of events leads to killing of CD19-expressing cells."

Potency Testing (FDA SBRA): i) Cell viability; ii) anti-CD19 CAR expression; iii) redacted

References

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Product: Breyanzi (lisocabtagene maraleucel) **Year approved:** 2021

Sponsor: Juno Therapeutics

Description: CD19-directed genetically modified autologous T-cell immunotherapy

Indication: Adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified (including DLBCL arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B

Clinical Trial Structure: Single arm (with 3 dose cohorts) (compared to historical data for standard of care)

Efficacy Endpoints: Objective response rate as assessed by i) survival, ii) disease progression

MOA (Package Insert): "CAR binding to CD19 expressed on the cell surface of tumor and normal B cells induces activation and proliferation of CAR T-cells, release of pro-inflammatory cytokines, and cytotoxic killing of target cells."

Potency Testing (FDA SBRA): i) Redacted

References

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Product: Abecma (idecabtagene vicleucel) **Year approved:** 2021

Sponsor: Celgene

Description: B-cell maturation antigen (BCMA)-directed genetically modified autologous T-cell immunotherapy

Indication: Adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody

Clinical Trial Structure: Single arm (with 3 dose cohorts) (compared to historical data for standard of care)

Efficacy Endpoints: Objective response rate as defined by i) serum and urine protein electrophoresis and immunofixation, ii) serum immunoglobulins (IgG, IgM, and IgA), iii) serum free light chain assay, iv) serum chemistry for creatinine, v) radiographic assessment of bone lesions, vi) extramedullary plasmacytoma assessments [by positron emission tomography-computed tomography (PET-CT) or magnetic resonance imaging (MRI)], vii) bone marrow biopsy (CD138+ cells and BCMA expression)

MOA (Package Insert): "ABECMA is a chimeric antigen receptor (CAR)-positive T-cell therapy targeting B-cell maturation antigen (BCMA), which is expressed on the surface of normal and malignant plasma cells. The CAR construct includes an anti-BCMA scFv-targeting domain for antigen specificity, a transmembrane domain, a CD3-zeta T-cell activation domain, and a 4-1BB costimulatory domain. Antigen-specific activation of ABECMA results in CAR-positive T-cell proliferation, cytokine secretion, and subsequent cytolytic killing of BCMA-expressing cells."

Potency Testing

- *FDA SBRA:* i) Redacted
- *EMA Assessment Report:* Interferon-γ production by product upon stimulation with BCMA+ cells

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Product: Carvykti (ciltacabtagene autoleucel) **Year approved:** 2023

Sponsor: Janssen Biotech

Description: B-cell maturation antigen (BCMA)-directed genetically modified autologous T-cell immunotherapy

Indication: Adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Survival and objective response rate (as defined by negative response for disease evaluations: i) myeloma protein measurements in serum and urine; ii) serum calcium corrected for albumin; iii) bone marrow examination; iv) skeletal survey; v) documentation of extramedullary plasmacytomas)

MOA (Package Insert): "CARVYKTI is a BCMA-directed, genetically modified autologous T-cell immunotherapy, which involves reprogramming a patient's own T-cells with a transgene encoding a chimeric antigen receptor (CAR) that identifies and eliminates cells that express BCMA. The CARVYKTI CAR protein features two BCMA-targeting single-domain antibodies designed to confer high avidity against human BCMA, a 4-1BB co-stimulatory domain and a CD3-zeta (CD3ζ) signaling cytoplasmic domain. Upon binding to BCMA-expressing cells, the CAR promotes T-cell activation, expansion, and elimination of target cells."

Potency Testing (FDA SBRA): i) CAR expression from viable T-cells; ii) redacted

References

- Summary Basis for Regulatory Action - Carvykti, FDA, 2023. Accessed April 25, 2023: https://www.fda.gov/vaccines-blood-biologics/carvykti
- Package Insert – Carvykti, FDA, 2023. Accessed April 25, 2023: https://www.fda.gov/vaccines-blood-biologics/carvykti

Product: Hemacord **Year approved:** 2011

Sponsor: New York Blood Center

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cell number; ii) viability of CD45+ cells; iii) viable CD34+ cell count; iv) Colony forming unit (CFU)

References

- Summary Basis for Regulatory Action - Hemacord, FDA, 2011. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hemacord-hpc-cord-blood
- Package Insert - Hemacord, FDA, 2011. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hemacord-hpc-cord-blood

Product: HPC Cord Blood - Clinimmune Labs **Year approved:** 2012

Sponsor: University of Colorado Cord Blood Bank

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cells; ii) viability of total nucleated cells; iii) viable CD34+ cell count; iv) redacted

References

- Summary Basis for Regulatory Action, HPC Cord Blood - Clinimmune Labs, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood
- Package Insert - HPC Cord Blood - Clinimmune Labs, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood

Product: Ducord **Year approved:** 2012

Sponsor: Duke University School of Medicine, Carolinas Cord Blood Bank

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoint: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted; v) redacted

References

- Summary Basis for Regulatory Action - Ducord, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/ducord-hpc-cord-blood
- Package Insert - Ducord, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/ducord-hpc-cord-blood

Product: HPC Cord Blood – LifeSouth **Year approved:** 2013

Sponsor: LifeSouth Community Blood Centers

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted

References

- Summary Basis for Regulatory Action, HPC Cord Blood - LifeSouth, FDA, 2013. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-lifesouth
- Package Insert - HPC Cord Blood - LifeSouth, FDA, 2013. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-lifesouth

Product: HPC Cord Blood – Bloodworks **Year approved:** 2016

Sponsor: Bloodworks

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cells (flow cytometry); iv) redacted

References

- Summary Basis for Regulatory Action, HPC Cord Blood - Bloodworks, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-bloodworks
- Package Insert - HPC Cord Blood - Bloodworks, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-bloodworks

Product: Allocord **Year approved:** 2013

Sponsor: SSM Cardinal Glennon Children's Medical Center

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood, migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cells; ii) viable nucleated cells; iii) viable CD34+ cell count; iv) colony forming units (CFU)

References

- Summary Basis for Regulatory Action - Allocord, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/allocord-hpc-cord-blood
- Package Insert - Allocord, FDA, 2013. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/allocord-hpc-cord-blood

Product: Clevecord **Year approved:** 2016

Sponsor: Cleveland Cord Blood Center

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total nucleated cell number; ii) viable nucleated cells; iii) viable CD34+ cell count; iv) redacted

References

- Summary Basis for Regulatory Action (SBRA), Clevecord, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/clevecord-hpc-cord-blood
- Package Insert - Clevecord, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/clevecord-hpc-cord-blood
- Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, Gerson SL, Lazarus HM, Cairo M, Stevens CE, Rubinstein P, Kurtzberg J. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med. 2001 Jun 14;344(24):1815-22. https://doi.org/10.1056/NEJM200106143442402
- Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, Marks DI, van Rood JJ, Scaradavou A, Horowitz MM. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004 Nov 25;351(22):2265-75. https://doi.org/10.1056/NEJMoa041276
- Cornetta K, Laughlin M, Carter S, Wall D, Weinthal J, Delaney C, Wagner J, Sweetman R, McCarthy P, Chao N. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). Biol Blood Marrow Transplant 2005;11(2):149-60. https://doi.org/10.1016/j.bbmt.2004.11.020

Product: HPC Cord Blood - MD Anderson **Year approved:** 2018

Sponsor: MD Anderson Cord Blood Bank

Description: Allogeneic cord blood hematopoietic progenitor cell therapy

Indication: Unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoints: Hematologic reconstitution (neutrophils, platelets, erythrocytes)

MOA (Package Insert): "Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature. The mature cells are released into the bloodstream, where some circulate and others migrate to tissue sites, partially or fully restoring peripheral blood counts and function, including immune function, of blood-borne cells of marrow origin."

Potency Testing (FDA SBRA): i) Total CD34+ count; ii) total nucleated cell count; iii) nucleated red blood cell; iv) viability of nucleated cells; v) viable CD34+ cells; vi) colony forming units assay (CFU)

References

- Summary Basis for Regulatory Action (SBRA), HPC Cord Blood - MD Anderson, FDA, 2018. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-md-anderson-cord-blood-bank
- Package Insert - HPC Cord Blood - MD Anderson, FDA, 2018. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/hpc-cord-blood-md-anderson-cord-blood-bank

Product: Provenge (sipuleucel-T) **Year approved:** 2010

Sponsor: Dendreon

Description: Autologous cellular immunotherapy (CD54+ cells activated with PAP-GM-CSF and suspended in Ringer's)

Indication: Asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer

Clinical Trial Structure: Two arm (Provenge compared to control autologous peripheral blood mononuclear cells that have not been activated)

Efficacy Endpoints: Survival

MOA (Package Insert): "While the precise mechanism of action is unknown, PROVENGE is designed to induce an immune response targeted against PAP, an antigen expressed in most prostate cancers. During ex vivo culture with PAP-GM-CSF, APCs take up and process the recombinant target antigen into small peptides that are then displayed on the APC surface."

Potency Testing (FDA SBRA): i) Number of CD54+ cells (flow cytometry); ii) increased expression of CD54 on the surface of antigen presenting cells after culture with PAP-GM-CSF (flow cytometry)

References

- Summary Basis for Regulatory Action – Provenge, FDA, 2010. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/provenge-sipuleucel-t
- Package Insert – Provenge, FDA, 2010. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/provenge-sipuleucel-t
- Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer 2009;115(16):3670-9. https://doi.org/10.1002/cncr.24429

Product: Laviv (Azficel-T) **Year approved:** 2011

Sponsor: Fibrocell Technologies

Description: Autologous fibroblasts from skin punch biopsy

Indication: Improvement of the appearance nasolabial fold wrinkles in adults

Clinical Trial Structure: Two-arm [Laviv compared to placebo (medium without fibroblasts)]

Efficacy Endpoint: Score improvements on the Evaluator Wrinkle Assessment Scale and the Subject Wrinkle Assessment Scale

MOA:

- *Package Insert:* **"**The mechanism by which LAVIV improves the appearance of nasolabial fold wrinkles is unknown."
- *FDA SBRA:* "The mechanism of action of azficel-T has not been demonstrated. However, each lot is tested to determine that the product consists of viable fibroblasts that produce collagen. The potency of azficel-T is determined by the combination of cell count, viability, identity as fibroblasts and collagen content. The rationale for the choice of these characteristics is based on the premise that fibroblast survival and collagen biosynthesis following injection of azficel-T are likely to be important factors for the improvement in appearance of nasolabial fold wrinkles."
- *Smith et al, 2012:* "The exact mechanism of action of injected autologous fibroblasts remains unknown. They may exert their effect through one of several mechanisms. These could include the direct secretion of increased amounts of collagen and elastin, the induced proliferation of native fibroblasts, the secretion of cofactors that otherwise augment the dermal milieu, or simply multiplication of the transplanted fibroblasts. Most likely is it a combination of several of these processes."

Potency Testing (FDA SBRA): i) Cell count; ii) cell viability; iii) collagen production by the cells

References

- Summary Basis for Regulatory Action - Laviv, FDA, 2011. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/laviv-azficel-t
- Package Insert - Laviv, FDA, 2011. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/laviv-azficel-t
- Smith SR, Munavalli G, Weiss R, Maslowski JM, Hennegan KP, Novak JM. A multicenter, double-blind, placebo-controlled trial of autologous fibroblast therapy for the treatment of nasolabial fold wrinkles. Dermatol Surg 2012;38(7):1234-43. https://doi.org/10.1111/j.1524-4725.2012.02349.x

Product: Gintuit **Year approved:** 2012

Sponsor: Organogenesis

Description: Allogeneic cultured keratinocytes and fibroblasts in bovine collagen

Indication: For topical application to a surgically created vascular wound bed in the treatment of adult mucogingival conditions (not intended to provide root coverage)

Clinical Trial Structure: Two arm [Gintuit compared to standard of care in the same patient (gingival graft taken from the subject's palate)]

Efficacy Endpoints: Ability to regenerate ≥2 mm of keratinized gingiva at 6 months

MOA:

- *Package Insert:*
    - "Gintuit does not function as a tissue graft. The mechanism of action by which Gintuit increases keratinized tissue at the treated site has not been identified."
    - "The active ingredients of GINTUIT are the allogeneic keratinocytes, allogeneic dermal fibroblasts, and bovine Type I collagen. In vitro studies have shown that GINTUIT secretes human growth factors and cytokines, and contains extracellular matrix proteins. Growth factors, cytokines, and extracellular matrix proteins are known to be involved in wound repair and regeneration."
- *FDA Briefing Document, BLA 125400, November 17, 2011:* "The Committee noted that the histology assay is a good measure of the structural integrity of the product, however, the assay is not an adequate, sensitive measure of biological activity. While the exact biological metric that is most appropriate for product potency remains unclear, the Committee discussed that it would be appropriate to include cytokine assays given the current understanding of product function."

Potency Testing (FDA SBRA): Histology (hematoxylin and eosin staining) with morphological assessments: epidermal coverage, epidermal development, basal cell layer keratinocyte viability, suprabasal cell layer keratinocyte viability, dermal thickness, fibroblast density, and matrix integrity

References

- Summary Basis for Regulatory Action - Gintuit, FDA, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/gintuit-allogeneic-cultured-keratinocytes-and-fibroblasts-bovine-collagen
- Package Insert – Gintuit, 2012. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/gintuit-allogeneic-cultured-keratinocytes-and-fibroblasts-bovine-collagen
- FDA Briefing Document, BLA 125400. Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) Meeting #54, FDA, November 17, 2011. Accessed May 2, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/gintuit-questions-and-answers
- McGuire MK, Scheyer ET, Nevins ML, Neiva R, Cochran DL, Mellonig JT, Giannobile WV, Bates D. Living cellular construct for increasing the width of keratinized gingiva: results from a randomized, within-patient, controlled trial. J Periodontol 2011;82(10):1414-23. https://doi.org/10.1902/jop.2011.100671

Product: MACI (matrix-induced autologous chondrocyte implantation) **Year approved:** 2016

Sponsor: Vericel

Description: Autologous cultured chondrocytes on porcine collagen membrane

Indication: Full-thickness cartilage defects of the knee

Clinical Trial Structure: Two arm [MACI compared to standard of care (microfracture)]

Efficacy Endpoints: Knee injury and Osteoarthritis Outcome Score (KOOS) at 2 yrs

MOA (Package Insert): "No clinical pharmacology studies have been conducted with MACI and a mechanism of action has not been established."

Potency Testing:

- *FDA SBRA: i)* Cell number; ii) redacted; iii) redacted
- *Rapko et al., 2007:* PCR measurement of aggrecan gene expression

References

- Summary Basis for Regulatory Action - MACI, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/maci-autologous-cultured-chondrocytes-porcine-collagen-membrane
- Package Insert – MACI, FDA, 2016. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/maci-autologous-cultured-chondrocytes-porcine-collagen-membrane
- Rapko S, Parker A, Mortelliti C, Duguay SJ. #P192 - Aggrecan gene expression as a potency marker for matrix-induced autologous chondrocyte implantation (MACI). Osteoarthritis and Cartilage 15, Supplement B, page B136, 2007. https://doi.org/10.1016/S1063-4584(07)61547
- Saris D, Price A, Widuchowski W, Bertrand-Marchand M, Caron J, Drogset JO, Emans P, Podskubka A, Tsuchida A, Kili S, Levine D, Brittberg M. SUMMIT Study Group. Matrix-applied characterized autologous cultured chondrocytes versus microfracture: two-year follow-up of a prospective randomized trial. Am J Sports Med 2014, 42:1384-94. https://doi.org/10.1177/0363546514528093

Product: Stratagraft **Year approved:** 2021

Sponsor: Stratatech

Description: Allogeneic cultured keratinocytes and dermal fibroblasts in murine collagen

Indication: Deep partial-thickness thermal burns

Clinical Trial Structure: Two arm (Stratagraft compared to standard of care (autograft) in comparable wound sites of the same patient)

Efficacy Endpoints: i) Difference in the percent area of the treatments that required autografting by 3 months; ii) durable wound closure

MOA (Package Insert): "In vitro studies have shown that Sratagraft secretes human growth factors and cytokines and contains human ECM proteins. Growth factors, cytokines, and ECM are known to be involved in wound repair and regeneration."

Potency Testing (FDA SBRA): Redacted

References

- Summary Basis for Regulatory Action - Stratagraft, FDA, 2021. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/stratagraft
- Package Insert – Stratagraft, FDA, 2021. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/stratagraft
- Gibson ALF, Holmes IV JH, Shupp JW, Smith D, Joe V, Carson J, Litt J, Kahn S, Short T, Cancio L, Rizzo J, Carter JE, Foster K, Lokuta MA, Comer A, Smiell JM, Allen-Hoffmann BL. A phase 3, open-label, controlled, randomized, multicenter trial evaluating the efficacy and safety of StrataGraft1 construct in patients with deep partial-thickness thermal burns. Burns 2021;47:1024-1037. https://doi.org/10.1016/j.burns.2021.04.021

Product: Rethymic **Year approved:** 2021

Sponsor: Enzyvant Therapeutics

Description: Allogeneic processed thymus tissue

Indication: Congenital athymia

Clinical Trial Structure: Single arm (compared to historical data for standard of care)

Efficacy Endpoint: Survival

MOA:

- *Package Insert:* "Rethymic is intended to reconstitute immunity in patients who are athymic. The proposed mechanism of action involves the migration of recipient T-cell progenitors from the bone marrow to the implanted Rethymic slices, where they develop into naïve immunocompetent recipient T-cells. Evidence of thymic function can be observed with the development of naïve T-cells in the peripheral blood."
- *FDA SBRA:*
    - "Rethymic is intended to function as if it is a normal endogenous thymus. Its thymic endothelial cells recruit immature host T-cells (thymocytes) into the slices where they undergo further maturation and positive and negative selection, releasing into circulation immunocompetent naive T-cells that are capable of providing protection from infection."
    - **"**The proposed mechanism of action is migration of the recipient's bone marrow-derived T-cell progenitors into the thymic allograft where they are "educated" to produce immunocompetent T-cells that are tolerant of both donor and recipient tissues while maintaining the ability to respond to foreign antigens."

Potency Testing (FDA SBRA): Histology-based (for tissue organization, viability and retention of important cell types believed to be important for product function)

Potency Comments (FDA SBRA): "Histological evaluation, including for potency, by histology is reasonable for a tissue-based product, though is limited by sensitivity and variabilities inherent in the method."

References

- Summary Basis for Regulatory Action - Rethymic, FDA, 2021. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/rethymic
- Package Insert – Rethymic, FDA, 2021. Accessed April 27, 2023: https://www.fda.gov/vaccines-blood-biologics/rethymic
- Markert ML, Gupton SE, McCarthy EA. Experience with cultured thymus tissue in 105 children. J Allergy Clin Immunol 2022;149:747-57. https://doi.org/10.1016/j.jaci.2021.06.028

Product: Zynteglo (betibeglogene autotemcel) **Year approved:** 2022

Sponsor: Bluebird Bio

Description: Zynteglo consists of an autologous CD34+ cell-enriched population that contains the patient's own hematopoietic stem cells (HSCs) transduced ex vivo with the BB305 lentiviral vector (LVV) encoding β^{A-T87Q}-globin

Indication: For treatment of adult and pediatric patients with ß-thalassemia who require regular red blood cell (RBC) transfusions

Clinical Trial Structure: Single arm (compared to patient symptoms prior to treatment)

Efficacy Endpoint: Transfusion independence (TI) defined as weighted average hemoglobin (Hb) ≥ 9 g/dL without RBC transfusions for ≥ 12 months at any time after Zynteglo infusion

MOA (Package Insert): "Zynteglo adds functional copies of a modified β-globin gene into patients' hematopoietic stem cells (HSCs) through transduction of autologous CD34+ cells with BB305 LVV. After Zynteglo infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce RBCs containing biologically active β^{A-T87Q}-globin (a modified β-globin protein) that will combine with α-globin to produce functional adult Hb containing β^{A-T87Q}-globin (HbAT87Q). β^{A-T87Q}-globin can be quantified relative to other globin species in peripheral blood using high-performance liquid chromatography. β^{A-T87Q}-globin expression is designed to correct the β/α-globin imbalance in erythroid cells of patients with β-thalassemia and has the potential to increase functional adult HbA and total Hb to normal levels and eliminate dependence on regular pRBC transfusions."

Potency Testing (FDA SBRA): i) Vector copy number (qPCR); ii) percent LVV+ cells; iii) colony forming cells; iv) β^{A-T87Q}-globin quantitative protein expression; v) redacted; vi) redacted

References

- Summary Basis for Regulatory Action - Zynteglo, FDA, 2022. Accessed April 26, 2023: https://www.fda.gov/vaccines-blood-biologics/zynteglo
- Package Insert - Zynteglo, FDA, 2022. Accessed April 26, 2023: https://www.fda.gov/vaccines-blood-biologics/zynteglo
- Locatelli F, Thompson AA, Kwiatkowski JL, Porter JB, Thrasher AJ, Hongeng S, Sauer MG, Thuret I, Lal A, Algeri M, Schneiderman J, Olson TS, Carpenter B, Amrolia PJ, Anurathapan U, Schambach A, Chabannon C, Schmidt M, Labik I, Elliot H, Guo R, Asmal M, Colvin RA, Walters MC. Betibeglogene Autotemcel Gene Therapy for Non-β^0/β^0 Genotype β-Thalassemia. N Engl J Med 2022;386(5):415-427. https://doi.org/10.1056/NEJMoa2113206

Product: Skysona (elivaldogene autotemcel) **Year approved:** 2022

Sponsor: Bluebird Bio

Description: Skysona consists of an autologous CD34+ cell-enriched population, that contains the patient's own hematopoietic stem cells (HSCs), transduced ex vivo with the Lenti-D lentiviral vector (LVV) containing the ATP-binding cassette, sub-family D, member 1 (ABCD1) gene encoding the adrenoleukodystrophy protein (ALDP).

Indication: To slow the progression of neurologic dysfunction in boys 4-17 years of age with early, active cerebral adrenoleukodystrophy (CALD)

Clinical Trial Structure: Single arm (compared to historical data)

Efficacy Endpoint: Time from onset of symptoms to first major functional disability (MFD) or death (MFD defined as loss of communication, cortical blindness, requirement for tube feeding, total incontinence, wheelchair dependence, or complete loss of voluntary movement)

MOA (Package Insert): "Skysona adds functional copies of the ABCD1 cDNA into patients' hematopoietic stem cells (HSCs) through transduction of autologous CD34+ cells with Lenti-D LVV. After Skysona infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including monocytes (CD14+) capable of producing functional ALDP. Functional ALDP can then participate in the local degradation of very long chain fatty acids (VLCFAs), which is believed to slow or possibly prevent further inflammation and demyelination."

Potency Testing (FDA SBRA): i) Vector copy number (qPCR); ii) percent LVV+ cells; iii) percent ADLP+ cells; iv) redacted; v) redacted; vi) redacted

References

- Summary Basis for Regulatory Action - Skysona, FDA, 2022. Accessed April 26, 2023: https://www.fda.gov/vaccines-blood-biologics/skysona
- Package Insert - Skysona, FDA, 2022. Accessed April 26, 2023: https://www.fda.gov/vaccines-blood-biologics/skysona

Product: Omisirge (omidubicel) **Year approved:** 2023

Sponsor: Gamida Cell Ltd.

Description: Omisirge is a nicotinamide modified allogeneic hematopoietic progenitor cell (HPC) therapy derived from cord blood. Omisirge contains two cell fractions from the same allogeneic cord blood unit (CBU): (1) ex vivo cultured fraction (CF) of CD34+ cells that will engraft, and (2) a supportive non-cultured fraction (NF) of the non-selected CBU cells. The CF is a yellowish suspension of selected hematopoietic CD34+ progenitor cells ex vivo cultured with nicotinamide (NAM). In addition to the CD34+ progenitor cells, the CF consists of other cell populations, including lineage committed myelomonocytic cells, dendritic cells and granulocytes. The NF is a reddish suspension consisting of allogeneic, hematopoietic mature myeloid and lymphoid cells collected from the non-selected cells.

Indication: For use in adults and pediatric patients 12 years and older with hematologic malignancies who are planned for umbilical cord blood transplantation following myeloablative conditioning to reduce the time to neutrophil recovery and the incidence of infection

Clinical Trial Structure: Two-arm (compared to umbilical cord blood transplantation)

Efficacy Endpoint: i) time to neutrophil recovery following transplantation and ii) the incidence of Grade 2/3 bacterial or Grade 3 fungal infections through Day 100 following transplantation

MOA (Package Insert): "Omisirge is a nicotinamide (NAM) modified allogeneic hematopoietic progenitor cell therapy derived from cord blood used as an allogeneic stem cell donor source. Omisirge is manufactured utilizing a proprietary NAM based technology producing enriched HPCs. NAM technology overcomes the induction of accelerated proliferation, differentiation, cellular stress and signaling pathways that are typically activated when HPCs are removed from their natural environment. Ex-vivo culturing of cord blood derived HPCs in the presence of NAM leads to preservation of their stemness, homing to the bone marrow (BM) and retained engraftment capacity as demonstrated by rapid neutrophil engraftment and multi lineage immune reconstitution as observed in the clinical trials with Omisirge."

Potency Testing (FDA SBRA): CD34+ cell fold-increase

References

- Package Insert - Omisirge, FDA, 2023. Accessed April 26, 2023: https://www.fda.gov/vaccines-blood-biologics/omisirge
- Summary Basis for Regulatory Action - Omisirge, FDA, 2023. Accessed May 24, 2023: https://www.fda.gov/vaccines-blood-biologics/omisirge
- Lin C, Schwarzbach A, Sanz J, Montesinos P, Stiff P, Parikh S, Brunstein C, Cutler C, Lindemans CA, Hanna R, Koh LP, Jagasia MH, Valcarcel D, Maziarz RT, Keating AK, Hwang WYK, Rezvani AR, Karras NA, Fernandes JF, Rocha V, Badell I, Ram R, Schiller GJ, Volodin L, Walters MC, Hamerschlak N, Cilloni D, Frankfurt O, McGuirk JP, Kurtzberg J, Sanz G, Simantov R, Horwitz ME. Multicenter Long-Term Follow-Up of Allogeneic Hematopoietic Cell Transplantation with Omidubicel: A Pooled Analysis of Five Prospective Clinical Trials. Transplant Cell Ther 2023;29(5):338.e1-338.e6. https://doi.org/10.1016/j.jtct.2023.01.031

Product: Lantidra (donislecel-jujn) **Year approved:** 2023

Sponsor: CellTrans Inc.

Description: Allogeneic pancreatic islet cellular therapy. LANTIDRA is the first marketed cell-based therapy made from deceased allogeneic donor pancreatic islets of Langerhans (cluster of cells within the pancreas) for the treatment of T1D in adults who are unable to approach target HbA1c because of current repeated episodes of severe hypoglycemia despite intensive diabetes management and education. LANTIDRA is composed of mixed populations of endocrine cells, including beta cells that produce insulin. At least 30% of the product is made of insulin-producing beta cells. Together, the cells composing LANTIDRA regulate blood glucose levels through secretion of hormones in response to glucose stimulation. LANTIDRA is a suspension of islet cells administered through the hepatic portal vein.

Indication: The treatment of adults with Type 1 diabetes who are unable to approach target HbA1c because of current repeated episodes of severe hypoglycemia despite intensive diabetes management and education.

Clinical Trial Structure: Single arm

Efficacy Endpoint: Insulin independence

MOA (Package Insert): "Pancreatic islets regulate blood glucose levels through secretion of multiple hormones in response to increases and decreases in blood glucose. Endocrine cells within pancreatic islets release insulin, glucagon, somatostatin, pancreatic peptide, and ghrelin. Insulin stimulates glucose uptake by peripheral tissues; glucagon mobilizes glucose from the liver into circulation; somatostatin inhibits both α- and β-cell secretions; pancreatic peptide inhibits pancreatic exocrine secretion; and ghrelin inhibits insulin secretion. The primary mechanism of action of LANTIDRA is believed to be secretion of insulin by infused(transplanted) β-cells."

Potency Testing (FDA Advisory Committee Meeting): i) Glucose Stimulation Index (GSI): ELISA (enzyme linked immunosorbent assay) quantification of insulin release in glucose stimulated islets; ii) Islet Yield: Dithizone (DTZ) stain and microscopic quantification; iii) Viability: SYTO 13 green/ethidium bromide staining and microscopic evaluation

References

- Package Insert - Lantidra, FDA, 2023. Accessed July 14, 2023: https://www.fda.gov/vaccines-blood-biologics/lantidra
- Summary Basis for Regulatory Action - Lantidra, FDA, 2023. Accessed August 9, 2023: https://www.fda.gov/vaccines-blood-biologics/lantidra
- FDA Cellular, Tissue and Gene Therapies Advisory Committee Meeting, AM Session Product Characterization - BLA 125734 (Lantidral; donislecel), April 15, 2021. Accessed July 17, 2023: https://www.fda.gov/advisory-committees/advisory-committee-calendar/cellular-tissue-and-gene-therapies-advisory-committee-april-15-2021-meeting-announcement-04152021
- Qi M, Kinzer K, Danielson KK, Martellotto J, Barbaro B, Wang Y, Bui JT, Gaba RC, Knuttinen G, Garcia-Roca R, Tzvetanov I, Heitman A, Davis M, McGarrigle JJ, Benedetti E, Oberholzer J. Five-year follow-up of patients with type 1 diabetes transplanted with allogeneic islets: the UIC experience. Acta Diabetol 2014;51(5):833-43. https://doi.org/10.1007/s00592-014-0627-6

Product: Casgevy (exagamglogene autotemcel) **Year approved:** 2023

Sponsor: Vertex Pharmaceuticals, Inc.

Description (Package Insert): "CASGEVY is a cellular gene therapy consisting of autologous CD34+ HSCs edited by CRISPR/Cas9-technology at the erythroid specific enhancer region of the BCL11A gene to reduce BCL11A expression in erythroid lineage cells, leading to increased fetal hemoglobin (HbF) protein production. CASGEVY is prepared from the patient's own HSCs, which are obtained via apheresis procedure(s). The autologous cells are enriched for CD34+ cells, and then genome edited ex vivo by introducing the CRISPR/Cas9 ribonucleoprotein (RNP) complex by electroporation. The guide RNA included in the RNP complex enables CRISPR/Cas9 to make a precise DNA double-strand break at a critical transcription factor binding site (GATA1) in the erythroid specific enhancer region of the BCL11A gene. As a result of the editing, GATA1 binding is disrupted and BCL11A expression is reduced. This reduction in BCL11A expression conversely results in an increase in gamma-globin expression and downstream fetal hemoglobin formation." "CASGEVY is provided as a single dose for infusion containing a suspension of CD34+ cells in one or more vials." "The minimum recommended dose of CASGEVY is 3×10^6 CD34+ cells/kg."

Indication: Treatment of patients aged 12 years and older with transfusion-dependent ß-thalassemia (TDT).

Clinical Trial Structure: Single arm

Efficacy Endpoint: The proportion of patients who did not experience any protocol-defined severe vaso-occlusive crises (VOCs) for at least 12 consecutive months within the first 24 months after CASGEVY infusion. VOCs were defined as: i) acute pain event requiring a visit to a medical facility and administration of pain medications (opioids or intravenous [IV] non-steroidal anti-inflammatory drugs [NSAIDs]) or RBC transfusions; ii) acute chest syndrome; iii) priapism lasting > 2 hours and requiring a visit to a medical facility; and iv) splenic sequestration.

MOA (Package Insert): "After CASGEVY infusion, the edited CD34+ cells engraft in the bone marrow and differentiate to erythroid lineage cells with reduced BCL11A expression. Reduced BCL11A expression results in an increase in γ-globin expression and HbF protein production in erythroid cells. In patients with severe sickle cell disease, HbF expression reduces intracellular hemoglobin S (HbS) concentration, preventing the red blood cells from sickling and addressing the underlying cause of disease, thereby eliminating VOCs."

Potency Testing (SBRA): i) On-target editing frequency (tracking of indels by decomposition, TIDE); ii) redacted; iii) redacted.

References

- Package Insert - Casgevy, FDA, 2023. Accessed February 14, 2024: https://www.fda.gov/vaccines-blood-biologics/casgevy
- Summary Basis for Regulatory Action - Casgevy, FDA, 2023. Accessed February 14, 2024: https://www.fda.gov/vaccines-blood-biologics/casgevy
- Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, Foell J, de la Fuente J, Grupp S, Handgretinger R, Ho TW, Kattamis A, Kernytsky A, Lekstrom-Himes J, Li AM, Locatelli F, Mapara MY, de Montalembert M, Rondelli D, Sharma A, Sheth S, Soni S, Steinberg MH, Wall D, Yen A, Corbacioglu S. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N Engl J Med. 2021 Jan 21;384(3):252-260. https://doi.org/10.1056/NEJMoa2031054

Product: Lyfgenia (lovotibeglogene autotemcel) **Year approved:** 2023

Sponsor: Bluebird Bio

Description (Package Insert): "Lygenia is a $\beta^{A\text{-}T87Q}$-globin gene therapy consisting of autologous CD34+ cells from patients with sickle cell disease containing hematopoietic stem cells (HSCs) transduced with BB305 LVV encoding $\beta^{A\text{-}T87Q}$-globin, suspended in cryopreservation solution. LYFGENIA is intended for one-time administration to add functional copies of a modified form of the β-globin gene ($\beta^{A\text{-}T87Q}$-globin gene) into the patient's own HSCs. LYFGENIA is prepared using the patient's own HSCs, which are collected via apheresis procedure(s). The autologous cells are enriched for CD34+ cells, then transduced ex vivo with BB305 LVV. The promoter, a regulatory element that controls the expression of the transgene selected for BB305 LVV, is a cellular (non-viral) promoter that controls gene expression specific to the erythroid lineage cells (red blood cells and their precursors). BB305 LVV encodes $\beta^{A\text{-}T87Q}$-globin."

Indication: Treatment of patients 12 years of age or older with sickle cell disease and a history of vaso-occlusive events (VOEs).

Clinical Trial Structure: Single arm

Efficacy Endpoint: Complete resolution of VOEs and severe VOEs between 6 months and 18 months after infusion of LYFGENIA. VOEs were defined as any of the following events requiring evaluation at a medical facility: i) an episode of acute pain with no medically determined cause other than vaso-occlusion, lasting more than 2 hours; ii) acute chest syndrome (ACS); iii) acute hepatic sequestration; or iv) acute splenic sequestration. Severe VOE (sVOE) were defined as either of the following events: i) VOE requiring a hospitalization or multiple visits to an emergency department/urgent care over 72 hours and receiving intravenous medications at each visit; or ii) priapism requiring any level of medical attention.

MOA (Package Insert): "LYFGENIA adds functional copies of a modified βA-globin gene (threonine [T] replaced with glutamine [Q] at position 87, T87Q or $\beta^{A\text{-}T87Q}$-globin) into patients' hematopoietic stem cells (HSCs) through transduction of autologous CD34+ cells with BB305 LVV. After LYFGENIA infusion, the transduced CD34+ HSCs engraft in the bone marrow and differentiate to produce red blood cells containing biologically active $\beta^{A\text{-}T87Q}$-globin that will combine with α-globin to produce functional Hb containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}). $\beta^{A\text{-}T87Q}$-globin can be distinguished from wildtype β^{A}-globin and from β^{S}-globin through reverse-phase high-performance liquid chromatography (RPHPLC) or ultra-high performance liquid chromatography (UPLC). HbA^{T87Q} has similar oxygen-binding affinity and oxygen hemoglobin dissociation curve to wild type HbA, reduces intracellular and total hemoglobin S (HbS) levels, and is designed to sterically inhibit polymerization of HbS thereby limiting the sickling of red blood cells."

Potency Testing (SBRA): i) Vector copy number; ii) redacted, iii) redacted; iv) redacted; v) redacted; vi) $\beta^{A\text{-}T87Q}$-globin quantitative protein expression.

References

- Package Insert - Lyfgenia, FDA, 2023. Accessed ed February 14, 2024: https://www.fda.gov/vaccines-blood-biologics/lyfgenia
- Summary Basis for Regulatory Action - Lyfgenia, FDA, 2023. Accessed February 14, 2024: https://www.fda.gov/vaccines-blood-biologics/lyfgenia
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Product: Amtagvi (lifileucel) **Year approved:** 2024

Sponsor: Iovance Biotherapeutics, Inc.

Description (Package Insert): AMTAGVI (lifileucel) is a tumor-derived autologous T cell immunotherapy comprised of a suspension of tumor-derived T cells for intravenous infusion. AMTAGVI is manufactured from resected patient tumor tissue prosected from one or more tumor lesions. Immune cells derived from a patient's tumor(s) are expanded in cell culture, washed, formulated as a cell suspension, and cryopreserved. The product must pass a sterility test before release for shipping as a frozen suspension in 1 to 4 patient-specific infusion bag(s) in individual protective metal cassettes. The product is thawed prior to administration back into the same patient. AMTAGVI is composed primarily of T cells of the CD4+T and CD8+T cell lineages. AMTAGVI may also contain monocytes and other lymphocytes, including B cells and NK cells. AMTAGVI may contain viable melanoma tumor cells from the original tumor tissue used to manufacture the product.

Indication: Indicated for the treatment of adult patients with unresectable or metastatic melanoma previously treated with a PD-1 blocking antibody, and if BRAF V600 mutation positive, a BRAF inhibitor with or without a MEK inhibitor.

Clinical Trial Structure: Single arm

Efficacy Endpoint: Objective response rate according to the RECIST guidelines (Response Evaluation Criteria In Solid Tumors), which is the fraction of patients demonstrating a complete response or partial response. Response is based on tumor load, which is assessed by measuring the longest axis of lesions identified during a baseline examination. The size of lesions can be measured by various means depending on the location: physical examination, chest x-ray, computed tomography, magnetic resonance imaging, ultrasound, etc. The RECIST criteria are as follows:

- *Complete response*—the disappearance of all target lesions;
- *Partial response*—at least a 30% decrease in the sum of the longest diameter of target lesions
- *Progressive disease*—at least a 20% increase in the sum of the longest diameter of target lesions
- *Stable disease*—neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease

MOA (Package Insert): "The specific mechanism of action of AMTAGVI (lifileucel) is unknown."

Potency Testing (SBRA): i) redacted, ii) redacted, iii) redacted, iv) redacted, v) dose (total viable cells), vi) redacted and vii) redacted.

References

- Package Insert - Amtagvi, FDA, 2024. Accessed ed February 20, 2024: https://www.fda.gov/vaccines-blood-biologics/amtagvi
- Summary Basis for Regulatory Action - Amtagvi, FDA, 2024. Accessed March 11, 2024: https://www.fda.gov/vaccines-blood-biologics/amtagvi
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Supplementary File 2: Definitions Cited in the Manuscript

Simon et al., Mechanism of Action, Potency and Efficacy: Considerations for Cell Therapies

Measurand: "the quantity or property intended to be measured"

> *Source:* International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd ed.; Joint Committee for Guides in Metrology (JCGM), 2012. Accessed June 1, 2023: https://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2012.pdf

Measurement: "process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity"

> *Source:* International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd ed.; Joint Committee for Guides in Metrology (JCGM), 2012. Accessed June 1, 2023: https://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2012.pdf

Mechanism of action (MOA): "pharmacologic action at the receptor, membrane, or tissue level"

> *Source:* Guidance for Industry and Review Staff - Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information - Good Review Practice. US Food and Drug Administration, 2009. Accessed June 1, 2023: https://www.fda.gov/media/77834/download

Mode of action: "the means by which a product achieves its intended therapeutic effect or action"

> *Source:* 21 CFR 3.2(k), Code of Federal Regulations. US National Archives. Accessed June 1, 2023: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-3/subpart-A/section-3.2

Potency: "The specific ability or capacity of the product, as demonstrated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result."

> *Source:* 21 CFR 600.3(s), Code of Federal Regulations, US National Archives. Accessed June 1, 2023: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-600/subpart-A/section-600.3#p-600.3(s)

Potency: "The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties."

> *Source:* International Conference on Harmonisation (ICH). ICH Harmonised Tripartite Guideline Specifications: Test Procedures And Acceptance Criteria For Biotechnological/Biological Products Q6B (ICH Q6B), Current Step 4 version, 1999. Accessed May 8, 2023: https://www.ich.org/page/quality-guidelines

Potency: "measure of the biological activity using a suitably quantitative analytical method, based on the attribute of the product which is linked to the relevant biological properties"

> *Source:* ISO 23033 - Biotechnology — Analytical methods — General requirements and considerations for the testing and characterization of cellular therapeutic products. International Organization for Standardization: Geneva, Switzerland, 2021.

Potency test: Tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3(s) of this chapter.

> *Source:* 21 CFR 610.10, Code of Federal Regulations, US National Archives. Accessed February 15, 2024: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-610/subpart-B/section-610.10

Efficacy: "Evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof."

> *Source:* Guidance for Industry - Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products. Food and Drug Administration, 1998. Accessed May 31, 2023: https://www.fda.gov/files/drugs/published/Providing-Clinical-Evidence-of-Effectiveness-for-Human-Drug-and-Biological-Products..pdf

Efficacy endpoint: "Measures intended to reflect the effects of a drug. They include assessments of clinical events (e.g., mortality, stroke, pulmonary exacerbation, venous thromboembolism), patient symptoms (e.g., pain, dyspnea, depression), measures of function (e.g., ability to walk or exercise), or surrogates of these events or symptoms."

> *Source:* Draft Guidance for Industry - Multiple Endpoints in Clinical Trials, US Food and Drug Administration, 2017. Accessed May 25, 2023: https://www.fda.gov/files/drugs/published/Multiple-Endpoints-in-Clinical-Trials-Guidance-for-Industry.pdf
>
> *Note: The term "efficacy endpoint" is used interchangeably with "clinical outcome".*

Clinical outcome: "Clinical outcomes directly measure whether people in a trial feel or function better or live longer."

> *Source:* Surrogate Endpoint Resources for Drug and Biologic Development, US Food and Drug Administration. Accessed May 25, 2023: https://www.fda.gov/drugs/development-resources/surrogate-endpoint-resources-drug-and-biologic-development
>
> *Note: The phrase "feels, functions or survives" is often used.*