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Machine learning models for optimizing Critical Quality Attributes (CQAs) in cell therapy release testing

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Abstract

Cell therapies represent one of the most promising frontiers in regenerative medicine, offering curative potential for conditions previously deemed untreatable. Yet, their complex biological nature and the high variability of living cells create unique challenges in ensuring consistent safety, potency, and efficacy. Critical Quality Attributes (CQAs) including viability, identity, purity, and functionality form the backbone of regulatory approval and therapeutic effectiveness, making their optimization central to reliable release testing. Traditional approaches, heavily dependent on manual assays and time-consuming protocols, often struggle to provide the speed, accuracy, and scalability required to meet patient needs and evolving regulatory demands. Machine learning (ML) has emerged as a transformative solution for CQA optimization by enabling data-driven, predictive, and adaptive release testing frameworks. By integrating diverse datasets ranging from flow cytometry and imaging to multi-omics and process analytics ML models can identify hidden patterns, predict CQA outcomes, and flag potential anomalies earlier in the manufacturing pipeline. Supervised learning models enhance predictive accuracy for potency and viability, while unsupervised and deep learning techniques reveal complex biological heterogeneity. This convergence not only accelerates release testing but also minimizes batch failures, reduces costs, and strengthens compliance through explainable and auditable AI systems. Moreover, ML-driven CQA optimization aligns with regulatory frameworks such as FDA and EMA quality-by-design (QbD) principles, enabling real-time monitoring and continuous process verification. By bridging computational intelligence with biomanufacturing science, machine learning redefines cell therapy release testing, advancing the field toward more resilient, scalable, and patient-focused production systems.

Keywords: Cell Therapy Manufacturing; Critical Quality Attributes (CQAS); Machine Learning; Release Testing; Quality-By-Design (QBD); Biomanufacturing Analytics

1. Introduction

1.1. Background: Rise of cell therapies and manufacturing complexities

Cell therapies have emerged as a groundbreaking class of advanced medicinal products, offering transformative treatments for conditions ranging from hematological malignancies to degenerative disorders [1]. Unlike traditional small molecules, these therapies rely on living cells that are expanded, modified, and reintroduced into patients, creating unique opportunities and unprecedented challenges. The complexity arises from their inherent biological variability, the sensitivity of cells to microenvironmental changes, and the intricate upstream and downstream processing steps

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required to maintain functionality and safety [2]. Manufacturing must accommodate patient-specific autologous therapies, which demand rapid, individualized workflows, as well as scalable allogeneic platforms, which prioritize batch consistency. Variability in raw materials, culture conditions, and process controls contributes significantly to risks in yield, potency, and stability [3].

Regulatory agencies have emphasized that manufacturing control is as important as clinical trial design, since deviations at this stage may compromise safety and efficacy [4]. The integration of advanced sensors (Table 2) and monitoring systems has become essential to track real-time fluctuations in pH, dissolved oxygen, and metabolite concentrations, which directly influence cell phenotype and therapeutic potential. This dynamic landscape underscores the necessity of robust control systems, paving the way for data-driven strategies to manage variability effectively [5].

1.2. Critical Quality Attributes (CQAs) and regulatory imperatives

Critical Quality Attributes (CQAs) are defined as measurable properties of a product that must remain within established limits to ensure safety, efficacy, and stability [6]. In the context of cell therapies, CQAs include cell identity, viability, purity, potency, and genetic stability. These attributes are influenced by upstream culture conditions, downstream purification, and cryopreservation processes. Their precise measurement is difficult, as conventional assays often require destructive sampling and provide delayed results. Figure 1 illustrates the interdependencies between process parameters and CQA variability, highlighting the complex network that must be stabilized to guarantee consistent therapeutic outcomes [7].

From a regulatory perspective, global agencies such as the FDA and EMA mandate rigorous monitoring and documentation of CQAs throughout the manufacturing life cycle [8]. The FDA's Chemistry, Manufacturing, and Controls (CMC) guidance emphasizes continuous process verification, aligning with the Quality by Design (QbD) paradigm. This approach shifts focus from endpoint testing to integrated process understanding. Table 3 provides examples of how machine learning has been used in related pharmaceutical contexts, illustrating a foundation for applying similar principles in cell therapy quality oversight [2]. Regulatory expectations thus not only demand compliance but also encourage innovation in how CQAs are monitored and optimized across production pipelines [3].

1.3. Role of machine learning in optimizing CQAs during release testing

Machine learning (ML) has emerged as a critical enabler for optimizing CQAs during release testing, where rapid and accurate assessments are vital. Traditional testing methods, while validated, often require significant time and manual intervention, delaying batch release and increasing costs [4]. ML-driven models, trained on high-dimensional sensor and process data, can predict quality outcomes in real time, reducing reliance on destructive assays. For instance, algorithms can learn correlations between spectral signatures from Raman sensors and potency assays, enabling non-invasive quality checks [1].

The predictive power of ML also enhances robustness by identifying hidden nonlinear patterns in data streams that conventional statistical tools cannot capture [5]. Anomaly detection models, discussed earlier in Table 1, can flag deviations before they compromise batch integrity, providing an early-warning system for operators [6]. Furthermore, reinforcement learning frameworks are being explored to dynamically adjust process parameters, optimizing viability and potency under varying culture conditions [7]. Such adaptive control aligns with regulatory imperatives for continuous verification while also addressing industrial scalability.

By bridging sensor-derived data (Table 2) with predictive analytics, ML transforms release testing from a reactive checkpoint into a proactive assurance mechanism [8]. This evolution demonstrates the necessity of precision-driven monitoring in ensuring reproducible therapeutic outcomes.

2. Conceptual foundations: CQAS and release testing in cell therapies

2.1. Defining CQAs in biomanufacturing and therapeutic relevance

Critical Quality Attributes (CQAs) are product properties that must be maintained within defined limits to ensure safety, efficacy, and stability in biopharmaceuticals [7]. In the context of cell therapies, CQAs extend beyond conventional chemical or physical characteristics to include biological metrics such as viability, proliferation capacity, genetic stability, and immunophenotype [9]. These parameters reflect not only the manufacturing process but also the therapeutic outcome, as subtle variations in cell phenotype can drastically alter clinical performance.

CQAs serve as the cornerstone of the Quality by Design (QbD) paradigm, where product development is guided by predefined objectives linked to patient outcomes [11]. For example, viability thresholds ensure that infused cells retain functional potency, while genetic stability checks prevent oncogenic transformation. Purity and identity assessments confirm that the desired therapeutic population has not been contaminated with residual host cells, microbial agents, or off-target subtypes [12]. These attributes represent measurable proxies for complex biological functions, bridging laboratory-based characterization with regulatory expectations.

The relevance of CQAs lies in their ability to safeguard translational integrity from research through to commercialization. Inconsistent or poorly defined CQAs increase the likelihood of adverse events, variability in therapeutic performance, and regulatory delays [10]. Establishing robust definitions also facilitates international harmonization of standards, enabling cross-border development of advanced therapies. As the industry matures, the integration of multi-omic and functional data into CQA frameworks is emerging, offering a richer view of quality that accounts for biological heterogeneity [13]. Thus, defining CQAs in biomanufacturing is not only a technical necessity but also a clinical imperative, ensuring that therapies meet both regulatory benchmarks and patient needs [8].

2.2. Traditional release testing methods and their limitations

Traditional release testing for cell therapies and biologics has relied heavily on laboratory-based assays designed to evaluate potency, purity, and safety prior to product release [9]. Methods such as flow cytometry, viability assays, sterility testing, and functional immune assays form the backbone of release testing regimes [7]. While effective in confirming that products meet minimum specifications, these techniques are inherently reactive, offering little insight into the dynamics of ongoing manufacturing processes [10].

A major limitation is the time-intensive nature of many tests. Sterility assays can take weeks to yield definitive results, delaying treatment delivery, which is particularly critical in autologous therapies where time sensitivity determines clinical outcomes [12]. Similarly, potency assays often require laborious culture and stimulation protocols, generating variability between laboratories and operators [13]. The reliance on destructive sampling also reduces available therapeutic material, an issue compounded by the small batch sizes typical of personalized therapies [11].

Another challenge lies in the incomplete coverage of process variability. Conventional release testing captures a snapshot of final product characteristics but fails to integrate the influence of upstream fluctuations. For instance, shifts in dissolved oxygen or pH levels during culture may influence potency, yet such changes remain invisible in endpoint assays [8]. As a result, release testing often provides retrospective confirmation rather than proactive assurance of quality.

The dependence on manual interpretation further exacerbates limitations. Flow cytometry data, for instance, requires expert gating strategies that can differ significantly across analysts, introducing subjective bias [7]. This variability is particularly problematic in regulatory submissions, where reproducibility and standardization are paramount.

Additionally, regulatory authorities are increasingly emphasizing continuous process verification, which requires a more holistic understanding of manufacturing performance [9]. Traditional methods, though established, lack the granularity and predictive capacity to meet these expectations. Emerging challenges in global supply chains and the rapid scaling of allogeneic platforms further highlight the inadequacy of conventional systems [10].

Collectively, these limitations underscore the urgency of adopting more sophisticated tools that can provide real-time, predictive insights into CQA dynamics. Such advancements would enable earlier detection of anomalies, reduce reliance on destructive testing, and accelerate the safe release of therapies [13].

2.3. Systems-based approach: linking CQAs to product safety, efficacy, and potency

A systems-based approach reframes CQAs not as isolated parameters but as interconnected elements within a broader biological and manufacturing network [12]. This perspective acknowledges that therapeutic success is shaped by the interplay of viability, purity, genetic stability, and potency, which together form the foundation of patient outcomes [11]. By considering CQAs in relation to process parameters and clinical endpoints, manufacturers can better predict therapeutic performance.

For example, shifts in metabolic profiles influence cell viability, which in turn impacts potency in cytotoxic lymphocyte therapies [7]. Similarly, small perturbations in genetic integrity can alter long-term persistence of infused cells, raising safety concerns [9]. These interdependencies highlight the inadequacy of linear testing strategies and support a framework where CQAs are monitored dynamically within a systems context.

Figure 1 presents a conceptual framework linking CQAs with cell therapy outcomes and release testing. The figure illustrates how variations in culture conditions propagate through CQA shifts to ultimately affect efficacy and safety [10]. By mapping these relationships, manufacturers gain a visual representation of quality risks and intervention points, facilitating proactive decision-making.

This systems-oriented lens also aligns with regulatory imperatives. Agencies emphasize the integration of product safety, efficacy, and potency into a unified risk-based assessment, which requires manufacturers to demonstrate comprehensive process understanding [13]. By linking CQAs directly to therapeutic outcomes, regulators can evaluate not only whether a product meets specifications but also whether it will consistently deliver intended clinical benefits [8].

Ultimately, this approach establishes the conceptual foundation for advanced analytics and machine learning integration. By embedding CQAs within systemic networks, ML models can be trained to capture nonlinear dependencies, enhance predictive monitoring, and support adaptive control strategies [9]. Thus, the rationale for transitioning toward ML-driven precision in release testing becomes both scientifically robust and regulatorily justified [12].

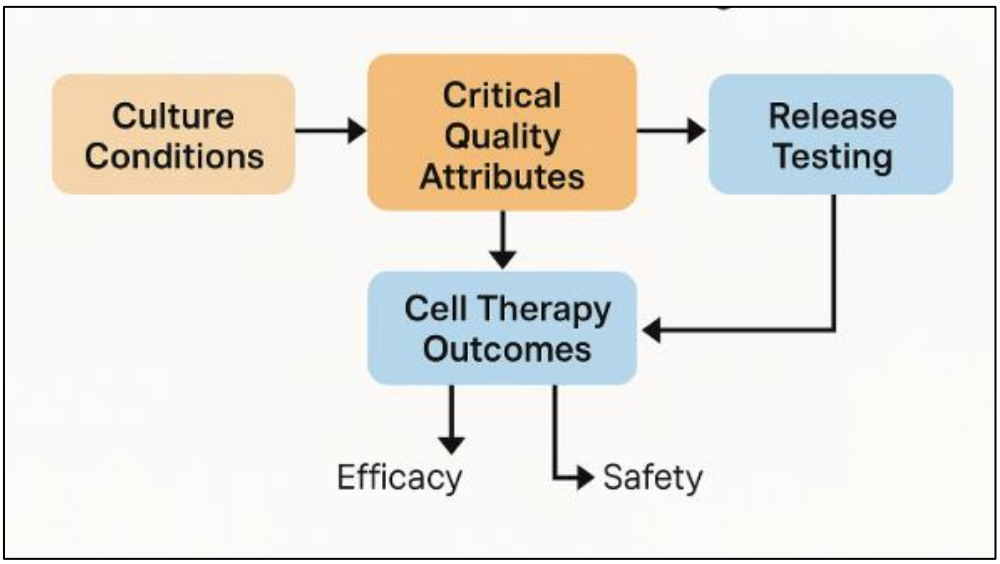

Figure 1 Framework linking CQAs with cell therapy outcomes and release testing

3. Challenges in cell therapy release testing

3.1. Variability in raw materials and donor-derived cells

One of the foremost challenges in cell therapy manufacturing lies in the variability of raw materials, particularly donor-derived cells, which form the basis of autologous and allogeneic products [12]. Unlike traditional biologics, where chemical precursors can be standardized, the intrinsic heterogeneity of living cells introduces unpredictable fluctuations in therapeutic potential. Factors such as donor age, genetic background, prior medical history, and even circadian rhythms can influence the proliferative and functional characteristics of harvested cells [15]. This biological diversity complicates efforts to establish consistent quality baselines across manufacturing batches.

Donor-to-donor variability also intersects with differences in raw material sourcing and processing. Serum lots, culture media, and reagents used in expansion can contribute additional layers of heterogeneity. Even when suppliers adhere to good manufacturing practices, subtle differences in nutrient composition or storage conditions affect cellular behavior [14]. This variability poses particular risks for therapies where potency depends on precise immune cell activity, such as chimeric antigen receptor T (CAR-T) treatments.

Beyond donor characteristics, pre-analytical handling introduces variability. Collection techniques, transport times, and cryopreservation protocols all alter viability and phenotype. Studies have demonstrated that delays in processing leukapheresis material significantly reduce expansion capacity and downstream potency [16]. In autologous workflows,

where each patient serves as both donor and recipient, such inconsistencies translate into unequal clinical responses, raising equity and safety concerns.

Regulatory agencies have highlighted the need for rigorous control of raw materials to mitigate these risks [13]. However, conventional methods remain limited in detecting subtle but clinically meaningful differences across donor sources. As more diverse populations are included in global therapy programs, inter-individual variability will only expand, placing strain on existing quality control strategies [17]. Addressing this challenge requires advanced analytical approaches that can integrate donor-specific factors with raw material characteristics, offering predictive insights into product quality. By systematically accounting for this variability, manufacturers can reduce batch failures, improve consistency, and enhance therapeutic predictability [18].

3.2. Time-sensitive processes and rapid testing requirements

Cell therapies are uniquely constrained by time, as manufacturing processes must be completed quickly to maintain product viability and deliver treatment within clinically relevant windows [14]. In autologous therapies, delays can compromise patient outcomes, while in allogeneic platforms, storage limitations reduce shelf life and utility [12]. Rapid, efficient testing is therefore critical, yet traditional release assays often fail to meet these demands.

Sterility testing exemplifies the tension between timeliness and rigor. Classical culture-based methods can take up to 14 days to confirm microbial safety, a timeframe incompatible with the urgency of delivering living-cell products [16]. While rapid microbial detection technologies exist, their sensitivity and regulatory acceptance remain limited, leaving manufacturers to balance safety with speed. Similarly, potency assays based on functional immune responses are inherently time-intensive, requiring multiple days of co-culture and stimulation [13]. These prolonged workflows introduce bottlenecks that delay therapy release.

Another challenge arises from the logistical constraints of individualized production. Autologous therapies necessitate parallel testing of small, patient-specific batches, magnifying the resource burden on laboratories [15]. Each test consumes valuable material from a limited supply, increasing the pressure to adopt non-destructive, rapid analytical approaches. Even automated testing systems often struggle with the throughput required to process dozens of concurrent patient lots under strict timelines [18].

Compounding the urgency is the fact that many patients receiving these therapies are critically ill, with limited time to wait for treatment [17]. Any delay in release not only affects manufacturing efficiency but also directly impacts survival and recovery. As a result, regulatory bodies have encouraged the adoption of innovative, science-based strategies to accelerate testing without compromising rigor [12].

Current approaches, however, remain constrained by the reliance on endpoint testing and linear data interpretation. Faster methods, such as flow-based microbial detection or high-throughput metabolic profiling, offer partial solutions but still lack comprehensive predictive power [16]. Addressing these limitations requires systems that can deliver real-time or near-real-time insights into product quality. Such capabilities would transform release testing from a bottleneck into a facilitator of rapid, reliable therapy delivery [14].

3.3. High-dimensional datasets and batch-to-batch inconsistencies

Cell therapy manufacturing generates vast, high-dimensional datasets from sensors, omics platforms, and quality control assays [13]. These data include temporal profiles of cell growth, metabolic fluxes, spectral signatures, and genomic stability checks, all of which together define the product's critical quality attributes. The challenge lies in effectively integrating and interpreting these complex datasets to detect batch-to-batch inconsistencies [17].

Traditional statistical tools are ill-suited to capture nonlinear patterns across multiple scales of data. For example, culture conditions such as dissolved oxygen, pH, and nutrient uptake rates interact in ways that defy linear modeling, yet these relationships are central to predicting potency and safety [12]. Batch inconsistencies often arise from subtle shifts in these variables, which may remain hidden until late-stage testing. As production scales from pilot to commercial volumes, the magnitude of these inconsistencies grows, amplifying the risk of therapeutic failure [16].

Complicating matters further, endpoint assays typically capture only a fraction of the available data, disregarding the continuous streams generated by advanced sensors. This fragmented approach prevents a holistic view of quality, leaving manufacturers vulnerable to unseen process drifts [18]. Moreover, inconsistencies between batches reduce confidence in therapeutic reproducibility, hindering regulatory approval and slowing market access [15].

Table 1 provides a comparative view of traditional versus machine learning–assisted release testing methods, illustrating how ML-based tools can integrate complex datasets, identify hidden variability, and provide predictive insights into quality control [14]. Unlike conventional methods, which react to observed deviations, ML algorithms proactively flag anomalies and correlate multidimensional data streams with CQAs, enabling earlier interventions.

Such predictive approaches are particularly relevant for allogeneic therapies, where large-scale batch manufacturing increases the impact of small deviations. Here, batch-to-batch consistency is paramount for regulatory acceptance and clinical trust [13]. Integrating ML into release testing pipelines offers the ability to reduce variability, enhance reproducibility, and align with the regulatory push toward continuous verification [17].

Ultimately, the challenge of interpreting high-dimensional datasets is not only a technical barrier but also a strategic opportunity. By adopting ML frameworks capable of extracting actionable insights from complex data, manufacturers can transform inconsistency from a liability into a source of predictive control [12].

Table 1 Comparison of Traditional vs. ML-Assisted Release Testing Methods

Dimension	Traditional Release Testing	ML-Assisted Release Testing
Testing Speed	Batch-based, often requiring days to weeks for assays and manual analysis.	Real-time or near-real-time predictions based on trained models, reducing turnaround significantly.
Data Utilization	Relies on predefined assays and a limited set of biomarkers; underutilizes high-dimensional datasets.	Integrates multi-omics, imaging, and process data simultaneously to capture complex biological variability.
Scalability	Difficult to scale due to dependence on physical assays, labor, and lab infrastructure.	Easily scalable once models are trained; computational scaling supports larger and more diverse datasets.
Variability Management	Limited ability to account for donor-to-door or batch-to-batch variability without extensive repeat testing.	Detects subtle patterns in variability, allowing predictive adjustments and proactive process control.
Error Detection	Manual review prone to human error; delayed anomaly identification.	Automated anomaly detection highlights outliers and data drift early, improving batch quality assurance.
Cost Implications	High due to repeated assays, consumables, and labor.	Lower long-term cost by reducing redundant assays and optimizing resource allocation.
Regulatory Alignment	Established but rigid, with defined assays meeting historical regulatory standards.	Emerging but promising; requires explainability and auditability frameworks for compliance acceptance.
Decision-Making	Based on threshold-driven manual decisions from test outputs.	Supports data-driven, adaptive decision-making with probabilistic confidence intervals.

4. Machine learning models for CQAS optimization

4.1. Supervised learning models for predictive CQAs analysis

Supervised learning remains one of the most widely applied machine learning (ML) paradigms for predictive analysis of Critical Quality Attributes (CQAs) in cell therapy manufacturing [16]. By training algorithms on historical datasets where inputs (e.g., culture conditions, sensor readings, and raw material characteristics) are paired with known outputs (quality outcomes such as potency or viability), supervised models learn to forecast CQAs in new manufacturing runs [18]. These models can help manufacturers anticipate deviations and intervene before a batch fails.

Linear regression and logistic regression have been foundational in early predictive modeling. However, more advanced methods like support vector machines (SVMs), random forests, and gradient boosting are increasingly favored due to their ability to capture nonlinear relationships between process variables and CQAs [20]. For example, random forest

models can identify which features such as pH fluctuations or nutrient consumption rates most strongly predict therapeutic potency, providing actionable insights for process control [22].

A critical advantage of supervised learning lies in its interpretability. Feature importance rankings allow manufacturers to understand not only what influences CQAs but also how those influences manifest, aligning with regulatory imperatives for transparency [17]. Such insights facilitate continuous process verification, offering a more proactive framework than traditional endpoint testing [21].

Nevertheless, supervised learning models depend heavily on the availability and quality of annotated training data [19]. Inconsistent labeling of potency assays, measurement errors, or small sample sizes can limit model generalizability. Moreover, biological variability across donors introduces noise that may reduce predictive accuracy. These challenges necessitate careful data curation, standardized assays, and robust validation practices to avoid misleading conclusions [24].

Supervised approaches are best viewed as part of a larger ecosystem, where predictive power complements real-time monitoring. Their integration into manufacturing pipelines demonstrates how historical data can be leveraged for future assurance of quality. As adoption grows, these models will increasingly support the alignment of manufacturing performance with patient outcomes, offering both predictive accuracy and regulatory compatibility [16].

4.2. Unsupervised learning for pattern recognition in cell populations

Unsupervised learning models provide powerful tools for uncovering hidden patterns in cellular data, where labels for CQAs may be incomplete or unavailable [18]. These methods group or reduce complex datasets into interpretable structures, offering insights into cellular heterogeneity and batch-level variability. Clustering algorithms such as k-means, hierarchical clustering, and density-based methods (DBSCAN) are widely used to identify subpopulations within cell therapy products that may influence potency or safety [19].

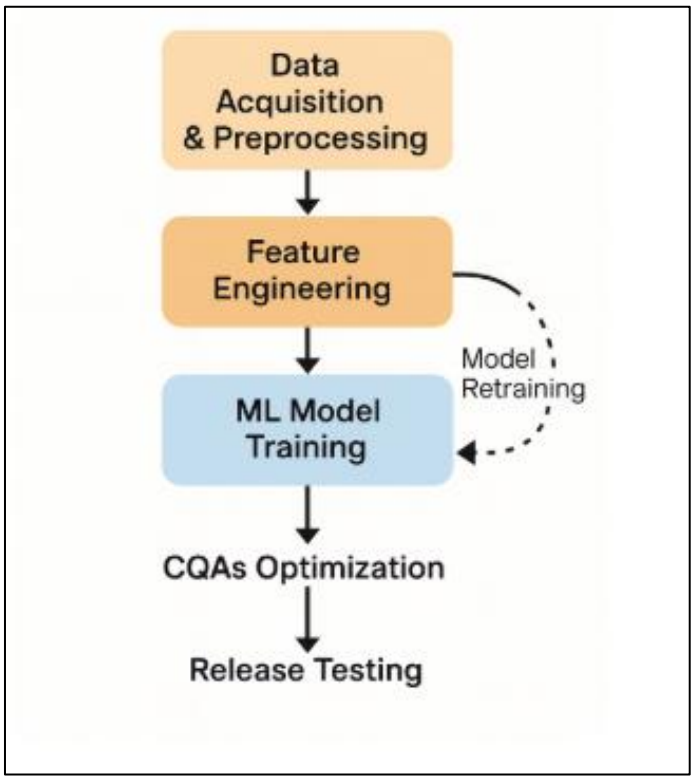

Figure 2 Workflow of ML models for CQAs optimization in release testing

For instance, unsupervised clustering of flow cytometry or single-cell RNA sequencing data has revealed phenotypic subgroups within T-cell populations that correlate with persistence and therapeutic success [23]. By distinguishing between functional and exhausted subpopulations, manufacturers can better anticipate product performance, even before clinical testing. Dimensionality reduction methods such as principal component analysis (PCA) and t-distributed

stochastic neighbor embedding (t-SNE) further assist by simplifying high-dimensional biological datasets into visualizable formats that highlight variability [20].

Another promising application of unsupervised learning is anomaly detection. Algorithms such as isolation forests or autoencoders trained in an unsupervised mode can detect outliers in sensor streams or batch-level data, flagging subtle process deviations that traditional monitoring might miss [16]. This aligns with the regulatory emphasis on early identification of risks rather than retrospective confirmation [21].

Figure 2 illustrates a conceptual workflow of ML models for CQA optimization, showing how unsupervised techniques complement supervised and deep learning by enabling discovery in unlabeled data [22]. By mapping cell populations and revealing hidden variability, these models reduce uncertainty and improve confidence in batch reproducibility.

Yet, limitations persist. Unsupervised algorithms are often sensitive to noise, and their outputs may vary depending on parameter selection or initial conditions [17]. Interpretation of clusters requires biological expertise, as statistical groupings do not always translate into clinically meaningful categories [19]. Despite these challenges, the capacity of unsupervised learning to extract hidden structures from complex cellular datasets makes it indispensable in advancing precision and robustness in cell therapy quality monitoring [24].

4.3. Deep learning models (CNNs, RNNs, autoencoders) for complex biological data

Deep learning offers unprecedented capabilities for modeling complex, nonlinear, and high-dimensional datasets characteristic of cell therapy manufacturing [23]. Convolutional neural networks (CNNs), recurrent neural networks (RNNs), and autoencoders are among the most frequently applied architectures, each suited to specific data modalities [20].

CNNs have been particularly successful in analyzing imaging data, such as microscopy images of cell morphology or tissue samples [18]. By automatically extracting spatial features, CNNs enable the detection of subtle phenotypic changes linked to CQAs, reducing reliance on manual inspection [16]. In quality control, CNNs have been applied to detect impurities or abnormal growth patterns in cell populations, providing a non-destructive method of monitoring product consistency [22].

RNNs, and especially their variants like Long Short-Term Memory (LSTM) networks, excel at handling temporal data streams. In manufacturing, RNNs can integrate sequential sensor readings such as nutrient consumption curves or metabolic fluxes to predict how process conditions evolve and impact CQAs [17]. This temporal awareness makes RNNs particularly relevant for real-time monitoring of dynamic bioprocesses.

Autoencoders, both shallow and deep, are widely used for dimensionality reduction and anomaly detection. By learning compact representations of high-dimensional omics or spectroscopic data, autoencoders can detect subtle deviations from expected patterns, flagging potential batch inconsistencies [19]. In release testing, these models support early interventions by highlighting deviations long before traditional assays confirm anomalies [21].

Despite their power, deep learning models face challenges of interpretability and data requirements [24]. Regulatory acceptance hinges on the ability to explain model outputs, yet deep networks are often perceived as “black boxes.” Efforts such as attention mechanisms, saliency maps, and explainable AI frameworks are therefore essential to align deep learning with compliance standards [23]. Additionally, these models demand extensive training datasets, which can be difficult to compile in tightly regulated and small-scale cell therapy contexts [16].

Deep learning’s ability to model complex biological systems makes it a cornerstone of next-generation CQA optimization strategies. When properly validated, these architectures can drive predictive, real-time, and scalable solutions that surpass the limitations of traditional analytics [18].

4.4. Hybrid and ensemble approaches for robust predictions

Hybrid and ensemble approaches combine multiple ML techniques to enhance robustness, accuracy, and resilience in predictive modeling of CQAs [20]. By integrating supervised, unsupervised, and deep learning methods, these approaches capture complementary strengths while mitigating individual weaknesses [17]. For instance, ensemble methods such as random forest and gradient boosting aggregate predictions across multiple learners, reducing variance and improving reliability in noisy datasets [22].

Hybrid approaches are particularly relevant in cell therapy, where diverse data types from sensor streams to omics profiles must be integrated for holistic quality assessment [21]. For example, a pipeline may use clustering to identify subpopulations, supervised learning to predict potency, and deep learning to monitor real-time sensor data, all within a unified framework [18]. Such integration enhances predictive power while providing regulators with layered evidence of quality assurance [16].

Although these methods can increase computational complexity, they offer a pathway toward resilient, generalizable models capable of handling biological variability and manufacturing inconsistencies [19]. Hybrid and ensemble frameworks thus represent a pragmatic approach for translating ML innovations into real-world release testing environments, supporting both predictive accuracy and regulatory compliance [24].

5. Data infrastructure and analytical workflows

5.1. multi-omics data integration (genomics, proteomics, metabolomics)

The integration of multi-omics data encompassing genomics, transcriptomics, proteomics, and metabolomics has become a cornerstone of precision biomanufacturing for cell therapies [23]. Each layer of omics provides complementary insights into product characteristics. Genomics identifies genetic modifications and stability, transcriptomics captures functional gene expression profiles, proteomics reflects dynamic protein activity, and metabolomics measures the biochemical environment that influences viability and potency [24]. By combining these dimensions, manufacturers can construct a holistic representation of Critical Quality Attributes (CQAs).

One of the critical benefits of multi-omics integration lies in its capacity to reveal hidden determinants of therapeutic quality [26]. For example, genomic markers may confirm the absence of oncogenic mutations, while proteomic signatures correlate with functional potency. When integrated, these markers generate robust predictive frameworks that strengthen release assurance. Moreover, metabolomic profiles provide real-time snapshots of cellular health, offering indicators of stress, exhaustion, or contamination [25].

However, integrating heterogeneous datasets is technically challenging. Omics platforms generate data with varying scales, noise distributions, and missing values. For instance, high-throughput sequencing yields vast genomic datasets, while proteomic measurements are often sparse and semi-quantitative [27]. Effective integration requires advanced computational pipelines capable of harmonizing modalities without losing biological context.

Supervised and unsupervised machine learning approaches have shown promise in this domain. Feature-level integration identifies shared patterns across omics, while model-level integration leverages ensemble strategies to merge predictions from separate datasets [28]. These strategies support robust characterization of CQAs and improve the ability to detect subtle deviations. Ultimately, multi-omics integration advances release testing by linking biological complexity with predictive analytics, aligning therapeutic quality with regulatory expectations [24].

5.2. Imaging and single-cell data in release testing

High-resolution imaging and single-cell profiling represent powerful modalities for enhancing release testing in cell therapy manufacturing [26]. Unlike bulk assays, which average responses across populations, single-cell approaches capture heterogeneity within cell products, offering greater insight into therapeutic potency and safety [23]. Imaging modalities, including brightfield microscopy, confocal imaging, and live-cell monitoring, provide visual confirmation of morphology, viability, and contamination [25]. When integrated with machine learning, these datasets can support automated, non-destructive assessments.

Single-cell RNA sequencing (scRNA-seq) has been especially impactful, enabling the identification of rare subpopulations that may influence long-term therapeutic outcomes [27]. For instance, exhausted or senescent cells can compromise potency, while highly proliferative subgroups may enhance persistence post-infusion. Clustering and dimensionality reduction methods applied to scRNA-seq allow manufacturers to map this heterogeneity, linking subpopulation dynamics directly to CQAs [28].

Imaging data, particularly when analyzed with convolutional neural networks, can further augment release testing by detecting subtle morphological variations not visible to human observers [24]. For example, CNNs can distinguish between activated and quiescent T cells based on shape and texture features, offering predictive insights into potency. These models provide real-time surveillance, minimizing delays associated with manual microscopy [26].

Nevertheless, challenges remain in standardizing imaging and single-cell platforms across manufacturing facilities. Differences in equipment calibration, reagent quality, and operator expertise can produce variability in results [23]. Additionally, single-cell datasets are inherently high-dimensional, requiring sophisticated preprocessing to reduce noise and extract meaningful biological signals [25]. Addressing these challenges is essential to ensure reproducibility, regulatory compliance, and integration into release testing pipelines.

The potential of imaging and single-cell data lies in its ability to provide non-destructive, high-resolution views of product quality. By linking cellular morphology, gene expression, and functional heterogeneity to CQAs, these modalities strengthen predictive monitoring and accelerate safe product release [27].

5.3. Preprocessing: feature selection, dimensionality reduction, and normalization

Before machine learning models can effectively analyze complex datasets, raw inputs must undergo rigorous preprocessing. This step ensures that heterogeneous data sources including omics, imaging, and sensor streams are harmonized into standardized formats suitable for predictive modeling [28]. Preprocessing plays a central role in improving model accuracy, reducing overfitting, and ensuring reproducibility [26].

Feature selection is a critical first step, designed to identify the most informative variables while discarding irrelevant or redundant features [24]. In genomics, this may involve selecting key variants associated with stability, while in metabolomics, it may focus on metabolites correlated with viability. By narrowing down variables, feature selection reduces computational burden and enhances interpretability [23].

Dimensionality reduction addresses the curse of dimensionality inherent in omics and imaging data. Methods such as principal component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE), and autoencoder-based embeddings enable the transformation of thousands of variables into lower-dimensional representations [27]. This process retains essential biological patterns while minimizing noise, facilitating downstream clustering and classification tasks.

Table 2 Data Types, Sources, and Preprocessing Strategies for ML-Driven CQA Analysis

Data Type	Source Examples	Preprocessing Strategies
Genomics	Whole-genome sequencing, RNA-seq, CRISPR screens	Quality filtering, normalization (e.g., TPM/RPKM), dimensionality reduction (PCA), feature selection (variant calling, gene expression signatures).
Proteomics	Mass spectrometry, Western blot, ELISA	Peak detection, noise reduction, spectral alignment, normalization across runs, log transformation, missing value imputation.
Metabolomics	LC-MS, GC-MS, NMR profiling	Baseline correction, peak alignment, scaling (Pareto, autoscaling), metabolite identification and annotation.
Imaging Data	Flow cytometry, immunofluorescence microscopy, digital pathology	Denoising, segmentation, feature extraction, dimensionality reduction (e.g., t-SNE, UMAP), normalization for illumination and contrast.
Single-Cell Data	scRNA-seq, CyTOF, single-cell ATAC-seq	Barcode demultiplexing, quality control (mitochondrial gene filtering), normalization, batch correction, clustering.
Bioprocess Sensor Data	pH, dissolved oxygen, temperature, bioreactor pressure	Signal smoothing, outlier removal, time-series normalization, resampling, feature engineering (derivatives, rates).
Clinical/Batch Records	Patient demographics, donor variability, production metadata	Data cleaning, categorical encoding, standardization of variables, handling missing records, metadata harmonization.

Normalization ensures that data from diverse sources are directly comparable. For example, proteomic intensities, metabolomic concentrations, and imaging features often exist on different scales. Normalization methods, including z-score transformation and quantile scaling, harmonize these ranges to prevent bias in model training [25]. Without

proper normalization, machine learning algorithms may erroneously prioritize artifacts over biologically relevant features.

Table 2 summarizes the data types, sources, and preprocessing strategies commonly applied in ML-driven CQA analysis, highlighting the importance of structured pipelines [26]. This table illustrates how preprocessing directly influences the reliability of predictions, bridging raw biological complexity with actionable insights.

Despite its importance, preprocessing introduces its own challenges. Overly aggressive dimensionality reduction can obscure rare but clinically significant patterns, while inappropriate normalization may distort biological signals [24]. Therefore, preprocessing must be carefully tailored to the context of cell therapy release testing, balancing standardization with preservation of biological variability [28].

Ultimately, preprocessing establishes the foundation for predictive analytics. By structuring raw data into robust, standardized inputs, it enables machine learning models to deliver accurate, interpretable, and regulatorily acceptable predictions of CQAs [23].

6. ML applications in anomaly detection and predictive release

6.1. Statistical vs. ML anomaly detection in cell therapy batches

Anomaly detection is a central requirement in cell therapy manufacturing, where early identification of deviations can prevent costly batch failures and safeguard patient safety [26]. Traditionally, statistical process control (SPC) methods such as control charts, Shewhart analyses, and process capability indices have been used to detect deviations from predefined limits. These approaches rely on assumptions of normality and linearity, which often fail in the biologically heterogeneous environment of cell culture [29]. As a result, subtle but clinically significant deviations may go undetected until final release testing.

Machine learning (ML) methods provide a more flexible framework by leveraging data-driven approaches that capture nonlinear and multivariate interactions [30]. Techniques such as isolation forests, support vector data description, and autoencoders identify anomalies without assuming predefined distributions. Unlike SPC, which flags deviations only when thresholds are breached, ML algorithms detect outliers based on deviations from learned patterns of normal process behavior [27].

A practical example involves dissolved oxygen fluctuations in bioreactors. SPC might fail to detect a gradual drift that remains within control limits, while ML models trained on multidimensional sensor data could flag the shift as anomalous, anticipating its downstream impact on potency [28]. This proactive approach supports real-time interventions, reducing risk of therapeutic loss.

Figure 3 illustrates a workflow for anomaly detection and predictive release using ML, demonstrating how real-time sensor streams feed into trained algorithms that continuously monitor batch integrity [31]. This visual framework emphasizes the distinction between reactive, threshold-based statistical detection and proactive, adaptive ML-based monitoring [26].

Despite these advantages, ML methods are not without challenges. Overfitting to historical noise, lack of interpretability, and dependence on high-quality training data remain barriers to regulatory adoption [32]. Nonetheless, by combining statistical rigor with ML adaptability, hybrid frameworks are emerging to balance interpretability with predictive accuracy [30]. Such systems align with the shift toward continuous verification and provide a strong case for the incorporation of ML anomaly detection into regulated workflows [33].

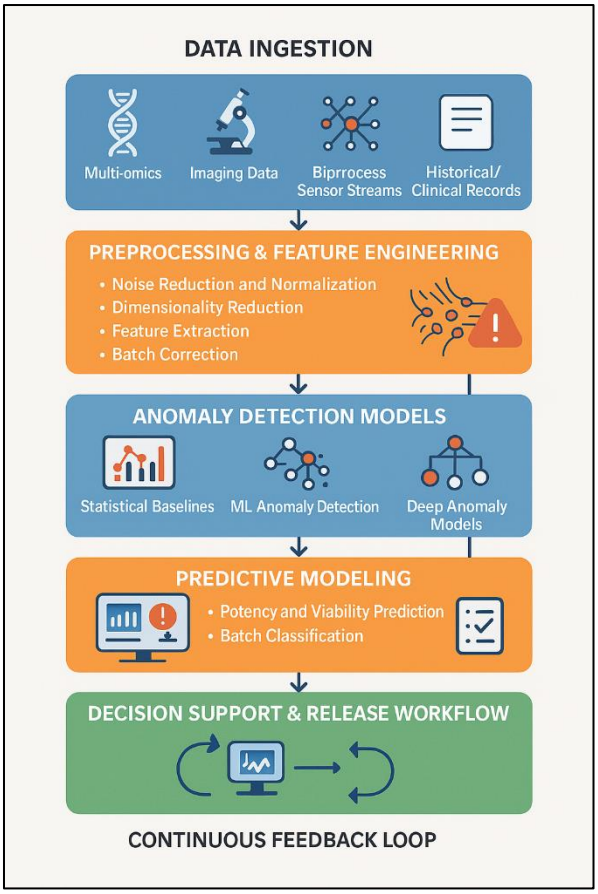

Figure 3 Anomaly detection and predictive release workflow using ML

6.2. Predictive modeling for potency and viability outcomes

Potency and viability represent two of the most critical CQAs in cell therapy release testing, directly influencing clinical efficacy and safety [29]. Predictive modeling offers an opportunity to estimate these outcomes in real time, reducing dependence on destructive, time-consuming assays [26].

Potency has traditionally been measured through functional assays such as cytokine release, cytotoxicity assays, or proliferation assays. While effective, these methods require significant time and biological material, often delaying release [30]. Machine learning can reduce these bottlenecks by correlating surrogate indicators, such as metabolic consumption rates, genomic signatures, or imaging features, with potency outcomes. Supervised models like random forests and gradient boosting have shown success in predicting potency with high accuracy, while deep learning architectures provide additional sensitivity to nonlinear interactions [27].

Viability outcomes, typically measured using staining or flow cytometry, can also benefit from predictive approaches. For example, CNNs trained on microscopy images can non-destructively predict viability percentages, offering rapid feedback without sacrificing therapeutic product [31]. RNN-based models trained on time-series sensor data, such as pH or nutrient consumption profiles, have also demonstrated predictive accuracy in forecasting viability across the culture cycle [28].

The predictive capacity of these models aligns with regulatory imperatives for continuous process verification [32]. By linking surrogate indicators to final CQAs, manufacturers can ensure batch release decisions are informed by high-frequency, real-time analytics rather than delayed laboratory assays. This approach reduces risk, increases efficiency, and supports scale-up for commercial manufacturing.

However, predictive modeling is only as robust as the data upon which it is trained [26]. Variability in assay standardization, incomplete labeling of potency endpoints, and donor-to-donor heterogeneity can introduce noise that compromises accuracy [33]. To address these issues, cross-validation, external benchmarking, and standardized data pipelines (as outlined in Table 2) remain critical [30].

By combining predictive modeling with advanced monitoring, manufacturers are not only accelerating release timelines but also strengthening reproducibility across batches. This synergy represents one of the most transformative benefits of ML integration in CQA optimization [29].

6.3. Integration of ML models with regulatory standards

The integration of ML models into regulatory frameworks is one of the most pressing challenges in the translation of data-driven manufacturing approaches [32]. Regulatory agencies such as the FDA and EMA have emphasized the need for transparency, reproducibility, and interpretability in quality monitoring systems [27]. This presents a tension: while ML models provide unparalleled predictive power, they are often criticized as "black boxes" that lack explainability.

Efforts to reconcile this tension include the development of explainable AI (XAI) tools, which generate interpretable insights alongside predictions [26]. For example, SHAP (Shapley Additive Explanations) values and attention mechanisms highlight which features contribute most to a model's output, offering regulators and manufacturers clarity about decision-making pathways [28]. Such tools make ML predictions auditable and align them with expectations for scientific justification.

Another critical issue is data governance. Regulators demand robust systems for data integrity, including version control, traceability, and audit trails [29]. ML pipelines must therefore include mechanisms to record model training processes, validation results, and performance monitoring, ensuring full compliance with Good Manufacturing Practices (GMP) [31]. Failure to meet these requirements can delay or prevent product approvals, regardless of technical efficacy.

International harmonization of standards also plays a key role. Agencies are working toward unified frameworks for digital quality monitoring, which would reduce regulatory fragmentation across regions [33]. By embedding ML validation into these frameworks, manufacturers gain confidence that their approaches will be accepted globally.

Importantly, regulatory agencies are not simply passive evaluators. Initiatives such as the FDA's Emerging Technology Program encourage proactive dialogue between innovators and regulators to define acceptable pathways for ML integration [30]. This collaborative approach ensures that regulatory frameworks evolve alongside technological innovation.

The successful integration of ML models into regulatory standards thus requires both technical validation and cultural adaptation. By combining transparency, governance, and harmonization, ML-enabled release testing can meet regulatory expectations while delivering enhanced quality assurance for patients [32].

6.4. Case of batch vs. continuous production monitoring

Cell therapy manufacturing has historically followed batch-based paradigms, where discrete lots undergo testing prior to release [26]. While effective for small-scale production, this model struggles to accommodate the growing demand for allogeneic therapies, which require larger volumes and reproducibility across batches [28]. Batch monitoring also introduces delays, as testing occurs only at defined endpoints rather than continuously.

Continuous production monitoring offers a more efficient alternative. By integrating ML-driven anomaly detection and predictive modeling, manufacturers can monitor quality in real time, adjusting process parameters dynamically to maintain optimal conditions [31]. This approach aligns with regulatory calls for continuous verification, offering greater assurance of consistency and safety [27].

However, the transition from batch to continuous monitoring presents challenges, including infrastructure requirements, data integration complexity, and regulatory adaptation [29]. Despite these hurdles, hybrid models are emerging, where batch frameworks incorporate elements of continuous monitoring for critical parameters.

Ultimately, continuous monitoring represents the future of scalable, efficient, and patient-centered cell therapy manufacturing [33]. By reducing reliance on endpoint testing, it accelerates release timelines and enhances confidence in therapeutic reproducibility, setting the stage for broader commercialization.

7. Case studies and practical applications

7.1. CAR-T therapies: ML applications in potency and viability testing

CAR-T therapies have emerged as one of the most transformative innovations in oncology, offering personalized treatment options for hematological malignancies [32]. However, their effectiveness depends heavily on ensuring that the final infused product meets stringent potency and viability thresholds. Traditional potency assays, such as cytokine release and cytotoxicity assays, are resource-intensive, slow, and often destructive. Machine learning (ML) models provide an alternative by leveraging real-time, high-dimensional datasets to predict these outcomes earlier in the manufacturing process [33].

Convolutional neural networks (CNNs) trained on microscopy images have demonstrated the ability to distinguish viable from apoptotic or senescent CAR-T cells without staining, offering a non-destructive viability test [35]. Similarly, recurrent neural networks (RNNs) applied to temporal metabolic data, such as glucose uptake or lactate production curves, can forecast functional capacity and persistence before clinical release [34]. These models reduce dependence on endpoint assays while providing continuous insights throughout culture.

Another promising application lies in integrating omics datasets. Transcriptomic and proteomic profiles can serve as surrogate markers for CAR-T potency, with supervised learning models linking these molecular signatures to clinical efficacy [36]. By combining multi-omics integration with imaging and process monitoring, ML frameworks create predictive pipelines that streamline release testing and improve patient safety.

Nevertheless, challenges persist. CAR-T therapies are autologous by nature, meaning donor-to-donor heterogeneity significantly affects data consistency [32]. Variability in T-cell expansion rates, transduction efficiencies, and phenotype distribution can reduce predictive accuracy unless models are trained on large, diverse datasets. Regulatory acceptance also remains cautious, with agencies requiring robust validation before ML-driven release decisions can replace or supplement traditional assays [37].

Despite these obstacles, evidence indicates that ML-enhanced testing reduces costs, accelerates release timelines, and provides more reliable measures of potency and viability. In doing so, it strengthens the therapeutic reproducibility of CAR-T therapies while aligning manufacturing workflows with regulatory imperatives for transparency and patient safety [35].

7.2. Stem cell therapies: variability management using ML

Stem cell therapies present a unique challenge in biomanufacturing due to the inherent heterogeneity of starting materials and the plasticity of stem cell states [34]. Unlike CAR-T therapies, which use relatively defined T-cell populations, stem cell products vary widely in differentiation status, lineage commitment, and genetic stability. These variations complicate the definition and monitoring of Critical Quality Attributes (CQAs) [36]. Machine learning offers tools to manage this variability by recognizing complex patterns in high-dimensional datasets and linking them to functional outcomes.

Unsupervised learning models such as clustering algorithms have been employed to classify subpopulations within stem cell cultures [32]. For example, hierarchical clustering of single-cell RNA sequencing data reveals differences in lineage trajectories, allowing manufacturers to identify subgroups that correlate with potency or risk of tumorigenicity [33]. These methods provide insights into culture heterogeneity that traditional bulk assays fail to capture.

Supervised approaches have also shown promise. Random forests and gradient boosting models trained on proteomic and metabolomic data can predict differentiation efficiency, guiding decisions about optimal harvesting times [37]. Similarly, deep learning models applied to imaging data can detect subtle morphological changes that signal early shifts in pluripotency or senescence [35]. By identifying these changes in real time, manufacturers can intervene to maintain quality and reduce batch failures.

The utility of ML in stem cell therapies extends to donor variability as well. Donor genetic background, epigenetic signatures, and even environmental factors can influence differentiation potential [36]. ML-driven predictive models integrate these donor-specific variables with process data, offering a means of tailoring protocols to individual sources while maintaining product consistency.

Challenges remain in ensuring reproducibility and regulatory compliance. Variability in single-cell platforms and imaging technologies can introduce noise, while overfitting risks reduce confidence in ML-driven predictions [34]. Nonetheless, ongoing improvements in preprocessing and standardization are enhancing the robustness of these models. Ultimately, ML provides a path toward reproducible, scalable stem cell therapy manufacturing by transforming heterogeneity from a liability into an actionable quality signal [32].

7.3. Industry adoption: commercial facilities and translational examples

The adoption of ML-based approaches in commercial cell therapy facilities marks a critical transition from research to practice [33]. Companies are increasingly integrating predictive models into manufacturing pipelines to accelerate release testing, reduce costs, and improve batch consistency [36]. Translational examples demonstrate how ML is reshaping the landscape of biomanufacturing.

One commercial facility specializing in CAR-T production has deployed ML algorithms to monitor bioreactor conditions in real time, linking oxygen consumption and nutrient uptake patterns to potency predictions [35]. These predictive insights allow for dynamic adjustments during culture, improving yields and reducing the likelihood of failed batches. In parallel, imaging-based ML pipelines have been used to automate quality checks, significantly reducing manual labor while enhancing reproducibility [37].

Stem cell therapy developers have also embraced ML frameworks. In one translational case, unsupervised clustering of single-cell datasets was integrated into decision-making pipelines, allowing operators to identify high-risk subpopulations before clinical release [32]. This approach not only improved safety profiles but also provided regulators with evidence of rigorous, data-driven monitoring.

Table 3 Case Study Summary – ML Optimization of CQAs in Different Therapy Types

Therapy Type	Case Study / Example	Optimized CQAs	ML Approach Used	Key Outcomes
CAR-T Cell Therapy	Prediction of in vivo potency using multi-omics data from manufacturing runs	Viability, cytokine release profiles, transduction efficiency	Supervised learning (random forests, XGBoost)	Early prediction of potency, reduced failed batch rates, improved consistency across donors.
Allogeneic Stem Cell Therapy	Batch variability analysis across donor-derived cell banks	Identity, purity, differentiation potential	Unsupervised clustering (k-means, hierarchical)	Detection of donor-specific variability; guided donor selection and pooling strategies.
iPSC-Derived Therapies	Imaging-based QC for pluripotency maintenance	Morphological integrity, pluripotency markers	Deep learning (CNNs for image classification)	Automated QC with >90% accuracy, reduced manual microscopy dependence.
Gene-Edited Therapies	On-target vs. off-target editing outcome prediction	Genetic integrity, editing efficiency, safety biomarkers	Ensemble models integrating CRISPR-seq data	Improved risk assessment; accelerated regulatory alignment for release testing.
NK Cell Therapies	Time-sensitive prediction of expansion success in bioreactors	Expansion kinetics, metabolic activity, viability	Recurrent neural networks (RNNs) for time-series modeling	Real-time monitoring of culture expansion, enabling early intervention in failing runs.

The advantages of ML adoption extend beyond technical outcomes to commercial viability. By reducing turnaround times, predictive pipelines enable faster delivery of therapies to patients, enhancing competitiveness in a rapidly growing market [34]. Furthermore, ML systems support scalability, a necessity as allogeneic therapies move toward large-scale manufacturing.

However, industry adoption is not without hurdles. The cost of integrating advanced data infrastructure, training personnel, and validating models for regulatory acceptance remains substantial [33]. Data security and governance also emerge as critical concerns, as ML pipelines often rely on sensitive genomic and clinical information [36].

Table 3 summarizes case study applications of ML optimization of CQAs across therapy types, highlighting how CAR-T, stem cell, and allogeneic products benefit from predictive analytics. The table illustrates common trends, including the use of imaging, omics integration, and anomaly detection to support release testing.

Together, these translational examples underscore the momentum behind ML adoption. By bridging laboratory innovation with industrial practice, they demonstrate how predictive analytics is becoming a central pillar of modern biomanufacturing, accelerating safe and reliable therapy delivery worldwide [37].

8. Governance, regulatory, and ethical considerations

8.1. FDA, EMA, and ICH guidelines for CQAs and ML adoption

Global regulatory agencies have recognized the importance of Critical Quality Attributes (CQAs) in assuring product safety, potency, and consistency in cell therapy manufacturing [36]. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have long emphasized quality-by-design (Qods) frameworks that place CQAs at the center of product development and release testing [37]. Similarly, the International Council for Harmonization (ICH) guidelines particularly ICH Q8, Q9, and Q10 define how manufacturers should manage quality risk, control strategies, and lifecycle management [39].

Machine learning (ML) adoption within this regulatory landscape introduces both opportunities and challenges. On one hand, ML enables real-time predictive monitoring, aligning with FDA's drive toward continuous process verification [38]. On the other hand, regulators remain cautious due to concerns over transparency, reproducibility, and data integrity [41]. Agencies increasingly expect manufacturers to validate ML pipelines with the same rigor applied to conventional assays, including version control and external benchmarking [40].

Recent pilot initiatives, including FDA's Emerging Technology Program and EMA's Adaptive Pathways, encourage early engagement between innovators and regulators to define acceptable standards for digital tools [42]. This proactive approach indicates a pathway forward, where ML-driven CQA monitoring can be harmonized with global regulatory expectations [43].

8.2. Explainability and auditability of ML models

One of the most significant barriers to ML adoption in regulated manufacturing is the so-called "black box" problem, where complex algorithms provide outputs without clear explanations of how decisions are made [37]. Regulatory authorities demand interpretability to ensure that predictions can be justified scientifically and audited for compliance [40]. Without explainability, even accurate models risk rejection in regulatory submissions [36].

Explainable AI (XAI) frameworks are emerging as essential tools in this context. Techniques such as Shapley Additive Explanations (SHAP), Local Interpretable Model-agnostic Explanations (LIME), and attention visualization help identify which features most strongly influence predictions [41]. For instance, when predicting CAR-T potency, SHAP values might highlight cytokine levels or metabolic fluxes as primary drivers, offering transparency to regulators and manufacturers alike [39].

Auditability extends beyond explainability to encompass full lifecycle traceability. Regulators expect manufacturers to demonstrate how models were trained, validated, and maintained over time [42]. This requires robust version control, metadata documentation, and reproducibility testing across independent datasets [38]. By embedding audit trails into ML pipelines, companies can align their systems with Good Manufacturing Practices (GMP), ensuring that models not only perform effectively but also meet the stringent requirements of regulatory oversight [43].

8.3. Ethical implications: patient safety and data bias

Ethical considerations represent another layer of complexity in ML adoption for cell therapy release testing [36]. At the heart of these concerns is patient safety, as predictive models influence decisions about whether therapies are released to clinics [38]. If models are trained on incomplete or biased datasets, they may fail to generalize across diverse patient populations, creating disparities in therapeutic outcomes [42].

Data bias is particularly concerning in autologous therapies, where donor-to-donor variability introduces heterogeneity into training datasets [40]. If underrepresented subgroups are insufficiently modeled, the resulting predictions could inadvertently disadvantage certain populations. Ethical frameworks therefore demand strategies for dataset diversification, bias detection, and continuous validation [41].

Another concern lies in overreliance on automation. While ML models enhance efficiency, they should complement, rather than replace, expert human judgment in release decisions [39]. This ensures a safeguard against systematic errors or unanticipated model failures.

Figure 4 illustrates a regulatory–technology–outcomes model for ML in cell therapy release testing, mapping how governance structures interface with technical frameworks to ensure patient-centered outcomes [43]. By embedding ethics into design, manufacturers can balance innovation with responsibility, ensuring that ML adoption advances both scientific progress and societal trust [37].

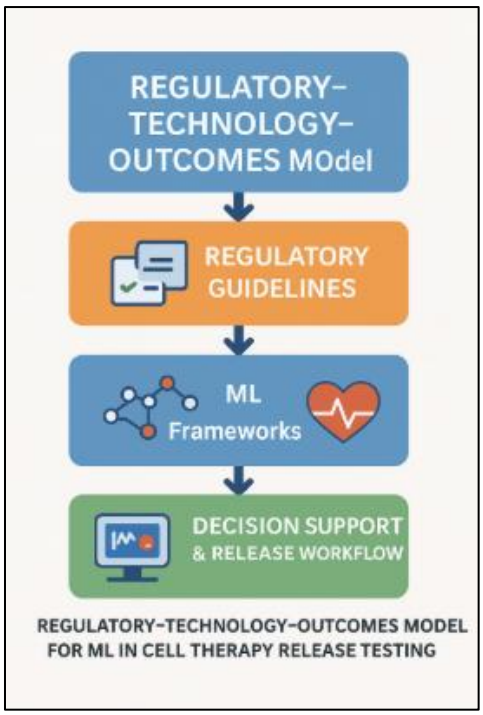

Figure 4 Regulatory–technology–outcomes model for ML in cell therapy release testing

9. Future directions and research gaps

9.1. Reinforcement learning for adaptive CQA optimization

Reinforcement learning (RL) has gained attention as a method for adaptively optimizing Critical Quality Attributes (CQAs) in real-time manufacturing environments [43]. Unlike supervised models, which rely on labeled datasets, RL agents learn by interacting with process environments and receiving feedback in the form of rewards or penalties [45]. This trial-and-error paradigm makes RL particularly suited to dynamic, nonlinear systems such as cell culture, where conditions shift continuously.

In cell therapy production, RL algorithms can adjust bioreactor parameters such as nutrient feed rates, dissolved oxygen, and agitation speed to maintain optimal quality conditions [47]. For example, an RL agent might learn that small increases in glucose supplementation prolong viability without triggering unwanted metabolic stress. Such adaptive strategies go beyond static control methods by tailoring interventions to evolving biological states [46].

The integration of RL into CQA monitoring also supports continuous verification, a priority for regulators seeking real-time assurance of therapeutic consistency [44]. However, challenges remain in ensuring stability and preventing unsafe exploration, particularly in high-stakes biomanufacturing [49]. Despite these concerns, RL demonstrates strong

potential to transform process control from reactive management into adaptive optimization, advancing both product reliability and patient safety [50].

9.2. Federated learning and data sharing across institutions

Federated learning (FL) provides a novel solution to one of the most pressing challenges in biomanufacturing: how to leverage diverse, multi-institutional datasets without compromising data privacy [46]. In this approach, models are trained collaboratively across institutions while data remains decentralized, ensuring that sensitive patient or genomic information never leaves its original source [44]. This paradigm is particularly valuable for rare cell therapies, where single facilities often lack sufficient data volume to train robust predictive models [47].

By aggregating model updates rather than raw data, FL enables collective intelligence while maintaining compliance with data protection regulations such as GDPR and HIPAA [48]. This makes it an appealing option for global cell therapy consortia seeking to harmonize predictive CQA frameworks across regions. Moreover, federated pipelines enhance model generalizability by capturing biological and process variability across diverse facilities [49].

Figure 5 outlines a roadmap of emerging ML applications, highlighting how federated learning fits within the broader landscape of data-driven CQA optimization [45]. By enabling collaboration without data exchange, FL bridges the gap between institutional silos and global regulatory harmonization. Ultimately, this ensures that predictive release testing frameworks benefit from collective expertise while respecting ethical and privacy constraints [43].

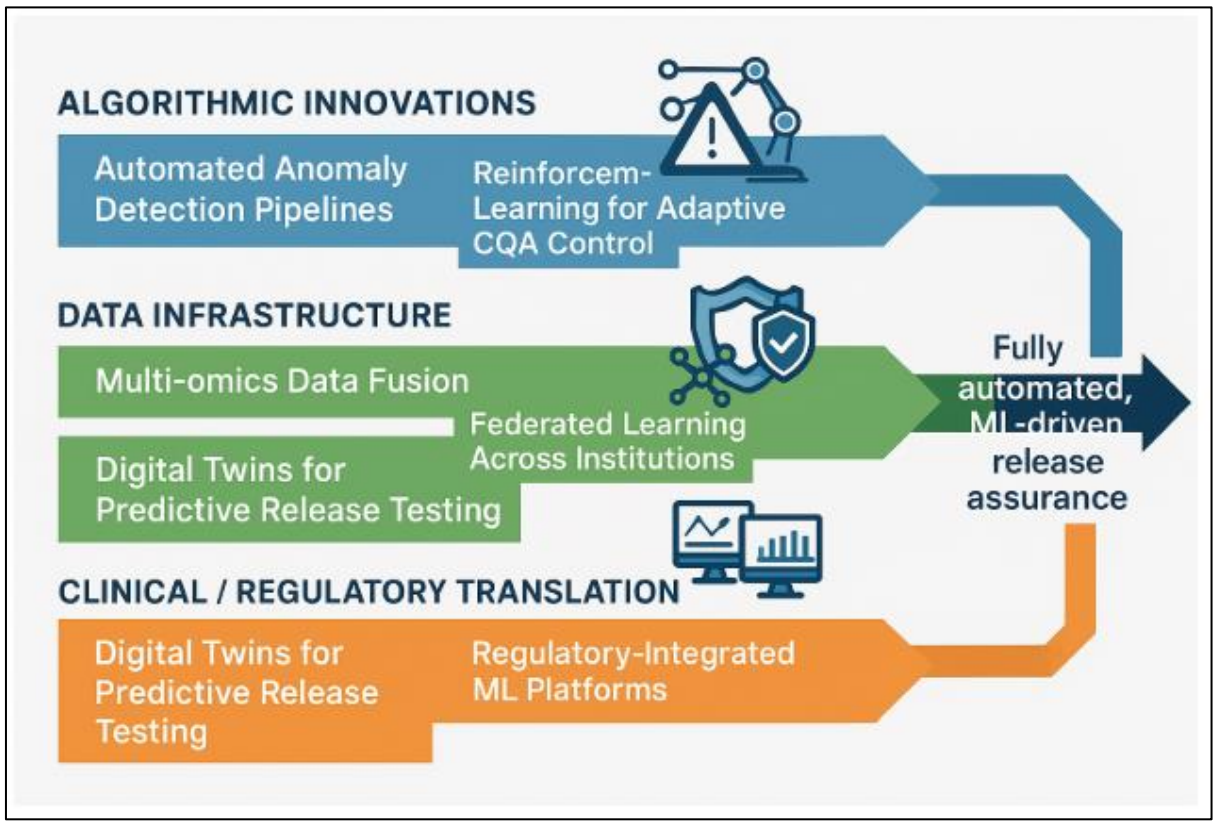

Figure 5 Roadmap of emerging ML applications in CQA optimization

9.3. Digital twins for predictive release testing

Digital twins' virtual replicas of physical manufacturing systems represent a frontier technology for predictive release testing in cell therapy [44]. By integrating real-time sensor data, process histories, and predictive ML models, digital twins provide a dynamic simulation environment that mirrors the behavior of actual production systems [47]. This allows manufacturers to test interventions, anticipate deviations, and optimize outcomes without risking real products.

In CQA optimization, digital twins can model how variations in culture conditions or raw material quality influence potency and viability outcomes [46]. For example, simulations may reveal how early nutrient depletion cascades into

reduced expansion capacity, enabling operators to intervene before therapeutic quality declines [49]. These insights extend beyond monitoring into predictive assurance, strengthening regulatory confidence in release decisions [45].

The adoption of digital twins also aligns with regulatory priorities for continuous verification, as they generate auditable, reproducible evidence of process control [48]. However, successful deployment requires significant infrastructure investment, including robust data integration, high-performance computing, and standardized modeling frameworks [50]. Despite these hurdles, digital twins hold transformative potential, enabling predictive, non-destructive release testing that accelerates product availability while safeguarding patient outcomes [43].

10. Conclusion

The application of machine learning (ML) to Critical Quality Attribute (CQA) optimization marks a transformative shift in the landscape of cell therapy manufacturing. Unlike traditional monitoring and release testing frameworks, ML-driven approaches provide real-time, adaptive insights into potency, viability, and product consistency. By integrating heterogeneous data streams ranging from multi-omics and imaging to high-frequency sensor inputs ML models allow manufacturers to identify subtle anomalies, predict therapeutic outcomes, and intervene proactively to safeguard quality. This capability addresses one of the most persistent challenges in cell therapy: balancing the complexity of biological systems with the demand for reproducible, scalable, and patient-safe products.

Equally important is the recognition that successful ML adoption extends beyond the models themselves. Data readiness, encompassing preprocessing, dimensionality reduction, and harmonization across platforms, provides the foundation upon which predictive pipelines can operate reliably. Likewise, governance frameworks ensure that ML integration remains transparent, auditable, and ethically grounded. Regulatory agencies are increasingly open to advanced digital tools, provided they meet rigorous standards of explainability, lifecycle management, and reproducibility. The alignment of ML systems with these governance requirements not only strengthens regulatory confidence but also enhances societal trust in emerging therapies.

The broader implication is clear: ML has the potential to redefine how cell therapies are tested, validated, and delivered to patients. Its predictive capacity shortens release timelines, reduces costs, and ensures higher consistency across batches and facilities. When combined with frameworks for continuous process verification, ML becomes a cornerstone of modern quality-by-design strategies, enabling therapies to reach patients faster without compromising safety.

Looking ahead, the adoption of ML in regulatory-aligned release testing should be viewed not as a technological option, but as a strategic imperative. Broader deployment across both academic and commercial facilities will accelerate standardization and generate shared evidence of success, further easing regulatory pathways. As ML systems evolve, integrating reinforcement learning, federated learning, and digital twins, the future of CQA optimization will move toward more dynamic, collaborative, and globally harmonized practices.

In conclusion, the path forward lies in embracing machine learning not merely as an analytical enhancement but as a foundational element of cell therapy manufacturing. Through integration of data, models, and governance, ML will play a defining role in delivering safe, effective, and scalable therapies to patients worldwide.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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