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Review Article

Serum alpha-fetoprotein kinetics as a mechanistic biomarker for rAAV-TK/GCV efficacy in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) remains a major fatal disease, and recombinant adeno-associated virus-thymidine kinase/ganciclovir (rAAV-tk/GCV) therapy is a newly discovered gene therapy with a unique mechanism of action for HCC. In the context of rAAV-tk/GCV treatment, serum alpha-fetoprotein (AFP), a commonly used clinical marker, is more of a static tumor-positive/negative biomarker and cannot dynamically and comprehensively reflect the pharmacodynamic response. Therefore, this article constructed a mechanistic model and integrates clinical and preclinical research data, proposing that changes in AFP kinetics are the result of four interacting processes: preferential elimination of proliferative tumor clones expressing high levels of AFP, heterogeneity of rAAV-tk transduction and the bystander effect of tk/GCV, treatment-induced liver tissue damage and regeneration in cirrhosis, and adaptive and innate immune responses. This article summarizes the typical AFP kinetics, including biphasic or uniphasic decline, lag phase, transient "rebound rise", low-level plateau phase, and secondary rise, and explains these characteristics in conjunction with reasonable physiologic mechanisms. Sources of uncertainty include impaired AFP clearance function or the basic regenerative state of the liver. Based on this, the article explores how early AFP kinetic parameters (e.g., percentage decline, slope, time to and depth of nadir) can serve as dynamic efficacy indicators and predictive biomarkers to facilitate dosage selection, trial enrichment and adaptive treatment. Finally, this article clarifies the main directions for translational research, including conducting follow-up clinical trials with intensive AFP sample collection, combining imaging detection and multi-omics analysis, and constructing of model-based decision-making models. It also proposes that the rational application of AFP kinetics based on its mechanism of action holds promise for significantly improving the optimization and clinical acceptance of rAAV-tk/GCV therapy in HCC.

Keywords: Hepatocellular carcinoma, serum alpha-fetoprotein kinetics, rAAV-tk/GCV suicide gene therapy, treatment response assessment, biomarker mechanism

Introduction

Hepatocellular carcinoma (HCC) remains a major global health challenge because of its high incidence, close association with chronic liver injury, marked biological heterogeneity, and persistently poor prognosis. HCC is considered one of the most frequent causes of cancer-associated mortality, and it typically occurs against a background of chronic liver disease and cirrhosis [1, 2]. Although surgery has the potential to cure a small portion of patients diagnosed at an early stage, most patients are diagnosed at an intermediate or advanced stage, resulting in unsatisfactory prognoses. In recent years, systemic therapies such as multikinase inhibitors, anti-angiogenic drugs, and immune checkpoint inhibitors have enriched treatment options and have played a certain modulating role in patient survival [3-5]. However, primary and acquired drug resistance are the most prevalent problems, treatment response rates remain unsatisfactory, and the biological heterogeneity of HCC also poses challenges for optimizing treatment regimens. Therefore, there is an urgent demand to explore

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novel treatment methods guided by mechanisms of action, as well as highly efficient biomarkers that can reflect clinical efficacy early, dynamically, and specifically. In addition to the limited duration of efficacy of existing treatments, the complexity of HCC is further exacerbated by the liver cirrhosis microenvironment, immune surveillance dysfunction, and inter-individual variability in carcinogenic drivers. These factors hinder accurate prediction of treatment response and rational treatment stratification. The current situation indicates that we not only need more selective treatment platforms, but also biomarkers capable of capturing biological responses in real time and in a targeted manner.

Against this background, gene-based therapies have garnered increasing attention as a potential precision-targeted treatment strategy for HCC [6-8]. Among emerging HCC treatment options, suicide gene therapy is particularly favored due to its reasonable tumor targeting. The recombinant adeno-associated virus (rAAV), carrying the herpes simplex virus thymidine kinase (HSV-tk) gene, is a typical example of this technology platform: tumor cells infected with the thymidine kinase (tk) gene can convert the less toxic prodrug ganciclovir (GCV) into a phosphorylated metabolite, thereby inducing cell death [9]. In addition to direct cytotoxicity, the bystander effect can extend the killing effect to neighboring untransduced tumor cells. Increasing evidence indicates that suicide gene therapy using rAAV vectors can also induce immunogenic cell death and remodel the tumor microenvironment, and these vectors exhibit good safety, hepatotropism, and stable transgene expression characteristics [10, 11]. However, as rAAV-tk/GCV therapy progresses from preclinical models to clinical applications, there is an urgent need for sensitive and practical biological activity monitoring and treatment decision-making tools. Importantly, the appeal of the rAAV-tk/GCV technology platform lies not only in its tumor-selective cytotoxic potential but also in its capacity to integrate vector biology, transcriptional targeting, and immune remodeling into a unified treatment modality. For the highly heterogeneous HCC, this mechanism-oriented approach offers advantages over conventional systemic regimens, yet its successful translation relies on reliable assessment methods to determine whether the expected biological effects occur promptly and are clinically meaningful.

Among existing biomarkers, serum alpha-fetoprotein (AFP) remains the most widely used indicator in HCC diagnosis and treatment due to its ease of detection, low cost, and long history of clinical application. Currently, serum AFP is the most commonly used circulating biomarker in HCC diagnosis and treatment; in tumors that produce AFP, its level can simultaneously reflect tumor burden and pathophysiologic status [12, 13]. Traditionally, AFP has been used as a static parameter in diagnosis, risk stratification and post-treatment monitoring, but its low sensitivity and specificity limit its application value as an independent biomarker. In recent years, research has begun to focus on AFP kinetics, namely the magnitude, trend, and timing of AFP changes during treatment, using it as a dynamic indicator for assessing treatment response and disease progression [14, 15]. In several treatment modalities, early AFP decline and characteristic AFP kinetic changes are associated with radiographic response and clinical prognosis, suggesting that AFP kinetics could be a low-cost, minimally invasive biomarker for real-time monitoring of treatment efficacy. Increasingly, studies no longer view AFP solely as a conventional tumor marker, but rather consider its dynamic changes to reflect the evolving biological interplay among tumor burden, treatment-induced cell death, inflammatory remodeling, and host response. This kinetic perspective is particularly important in treatment scenarios where antitumor effects are not immediate or solely cytoreductive, since these effects operate through a temporally progressive process, and their changes may precede or be inconsistent with radiographic changes.

This concept is particularly noteworthy in treatment based on rAAV-tk/GCV therapy. This approach is highly persuasive in HCC cases treated with rAAV-tk/GCV: suicide gene vectors regulated by AFP or other tumor-selective enhancers can promote tumor cell death and immune regulation mediated by rAAV-tk/GCV, and the resulting dynamic enhancement of AFP expression characteristic of rAAV-tk/GCV can be maintained in immunosuppressive cell responses [16]. Although this theoretical basis

is very solid, the research evidence on suicide gene therapy for HCC, as well as the relevant evidence on the dynamic changes of AFP under different treatment modalities, are mostly developing in parallel, and few studies have integrated the two into a coherent translational research framework. This research gap is of great significance because a deep understanding of the mechanism of AFP kinetics can help optimize early efficacy assessment, accurately screen patients, and allow for optimization of biomarker-guided rAAV-tk/GCV treatment strategies. This study reviews existing literature on rAAV-tk/GCV suicide gene therapy for HCC and the dynamic changes in serum AFP under various treatment regimens for HCC. A model framework is constructed, in which serum AFP kinetics can serve as a biomarker for clinical efficacy and mechanism of action, providing guidance for the rational development of rAAV-tk/GCV-based therapeutic intervention.

Serum AFP kinetic characteristics after rAAV-tk/GCV treatment: a comprehensive analysis of clinical evidence

Although rAAV-based suicide gene therapy theoretically holds promising potential for HCC, sufficient clinical evidence is currently lacking regarding serum AFP kinetic characteristics associated with specific rAAV-tk/GCV treatment regimens. The vast majority of existing human studies on HSV-tk/GCV suicide gene therapy for HCC use adenovirus vectors (e.g., intratumoral injection of TK99UN, adjuvant ADV-TK therapy after transplantation), and continuous monitoring of AFP was not a pre-specified primary biomarker endpoint in such studies, resulting in relatively few related reports [17]. Similarly, rAAV-tk vectors carrying AFP promoters or hepatocyte-specific promoters, while demonstrating good tumor suppression effects in preclinical HCC tumor models, have not been used in AFP kinetic studies. Therefore, current understanding of normal AFP kinetics after rAAV-tk/GCV-targeted AFP gene therapy is largely based on three aspects: first, the biological mechanisms of AFP-related tumor burden reduction; second, preclinical studies of the HSV-tk system targeting AFP; and third, the richer AFP kinetics data in non-gene-treated AFP-positive HCC patients.

Even with these limitations, several highly reproducible AFP kinetic patterns can still be predicted - some of which have been observed incidentally and sporadically in a few cases and are supported by substantial analogous evidence from other anti-HCC therapy. In both local and systemic treatments, early AFP decline, normalization of AFP at key time points, and various delayed AFP kinetic changes (rapid decline, delayed decline, stabilization, and sustained increase) have repeatedly been shown to be associated with radiographic efficacy and patient survival. This pattern is particularly evident in a series of studies combining radiotherapy and immunotherapy: the proportion of early decreases in AFP and the return to normal levels are closely related to progression-free survival and overall survival, and the dynamic changes in AFP are superior to single numerical measurements [18-20]. Based on this framework, predictions for rAAV-tk/GCV treatment can be made as follows: First, in some patients, due to high viral transfection efficiency and significant bystander killing effect, AFP will exhibit a rapid, monophasic decrease; second, some patients will show a biphasic pattern, i.e., a brief increase in AFP followed by a sustained decrease, which may be caused by transient tumor lysis or inflammatory necrosis; third, some patients will exhibit a biological resistance pattern, with poor bystander killing effect and viral transfection efficacy. The mechanism of action consistent with the above hypothetical patterns is cytotoxicity and immunogenic cell death induced by HSV-tk/GCV, but this mechanism has not yet been quantitatively verified in a human cohort receiving rAAV-tk treatment.

However, limited clinical experience with suicide gene therapy indicates that, in cases of elevated baseline AFP levels, increasing the frequency of testing and employing standardized criteria can provide a low-cost, real-time alternative indicator reflecting the bioactivity of rAAV-tk/GCV. Future gene therapy research for HCC should incorporate high-density time-series AFP monitoring (e.g., weekly monitoring during prodrug therapy and monitoring at predetermined critical post-treatment time points). This will rigorously quantify experimental endpoints such as changes in tumor burden, dynamic range, and kinetic patterns, combining them with AFP molecular delivery and intratumoral tk expression, while simultaneously conducting imaging examinations and immune monitoring. Such datasets can distinguish

between non-pathologic AFP fluctuations and biochemical failure-related AFP spikes, helping to elucidate the dynamic changes in AFP kinetics with vector dosage and promoter selection, and clarifying the rationality of early treatment termination rules or dose escalation protocols based on AFP. Before relevant data are available, the clinical evidence for AFP kinetics after rAAV-tk/GCV treatment can only serve as a theoretical model derived by extrapolation based on the mechanism of action. This model is highly reasonable and draws heavily on similar research findings in HCC [21-24], but it has not yet been directly verified in specific rAAV-tk/GCV experiment.

Mechanistic analysis of AFP kinetics drivers

In HCC treated with rAAV-tk/GCV, serum AFP kinetics is a comprehensive result of multiple dynamic interactions, rather than a direct correlation with tumor volume. Circulating AFP levels at any given time point reflect the regional synthesis, cell release, and hepatic clearance of malignant and non-malignant hepatocytes, and gene therapy can perturb these processes to varying degrees [25, 26]. Mechanistically, rAAV-tk/GCV selectively targets proliferative, progenitor-like tumor cells - these cells typically highly express AFP - while more differentiated tumor subclones with low or no AFP expression disappear. These subclones are inherently less sensitive to S-phase-dependent cytotoxicity. Therefore, a sharp initial decrease in AFP often indicates the effective killing of AFP-rich proliferative tumor clones; while the inability of AFP to fully recover to normal, a gradual decline, or an early plateau suggests the presence of quiescent cancer stem cell-like cells, areas with poor tumor blood supply or extremely low transduction efficiency, and micrometastases. Even with significant lesion shrinkage visible on imaging, these sites will continue to produce residual AFP signals (**Figure 1**).

The pharmacokinetic characteristics of the carrier and drug, superimposed on the aforementioned intrinsic tumor determinants - capsid serotype, vascular accessibility, and the expression levels of receptors and co-receptors regulating capsid-tumor binding, and interstitial pressure, cause characteristic delays and inflection points in the AFP time-varying curve, introducing nonlinear characteristics [27]. Furthermore, due to the need to complete second-chain synthesis and transcriptional activation, there is usually a lag of several days in tk expression reaching its peak after carrier delivery. Clinically, this often manifests as an early plateau in the AFP curve - even though the molecular initiation process is already active, AFP remains relatively stable or experiences only slight fluctuations; however, once the tk/GCV-mediated killing effect takes effect, AFP decreases more rapidly. Through the bystander effect, phosphorylated GCV metabolites can be transferred between tk-positive and negative cells through gap junctions or vesicle efflux, exerting cytotoxic effects in tightly connected areas of tumor cells. When the transduced cell population reaches a critical density, it can trigger a sharp drop in AFP [28, 29]. On the other hand, during acute vascular injury, microthrombus formation, and necrosis, the release of pre-existing intracellular protein pools and stress-induced transcriptional activation can lead to a short-term increase in AFP. These phenomena often occur briefly at the initial stage of GCV treatment initiation, and their mechanisms are distinctly different from long-term biochemical progression [30-32].

The liver microenvironment in which most HCCs occur has a pathologic basis, which gives additional characteristics to the changes in AFP and essentially affects its dynamic performance, making it more difficult to interpret [33-35]. In cirrhotic or chronically inflamed livers, AFP is not a tumor-specific protein; during the liver tissue damage and repair phase, activated hepatic progenitor cells and regenerated hepatocytes can re-express AFP and produce non-tumor cytotoxicity, triggering massive apoptosis and compensatory regeneration of hepatocytes. This type of regeneration mechanism may lead to a low-amplitude increase or a gradual secondary increase in AFP, which does not necessarily mean tumor recurrence [36]. At the same time, cirrhosis, sinusoidal capillarization, and portosystemic shunting can interfere with the clearance process of AFP, prolonging its half-life, causing the decrease in serum AFP levels to lag behind the secondary decrease in tumor secretion [37]. Therefore, the rapid and significant decrease in AFP levels serves as a fast and sensitive indicator of effective

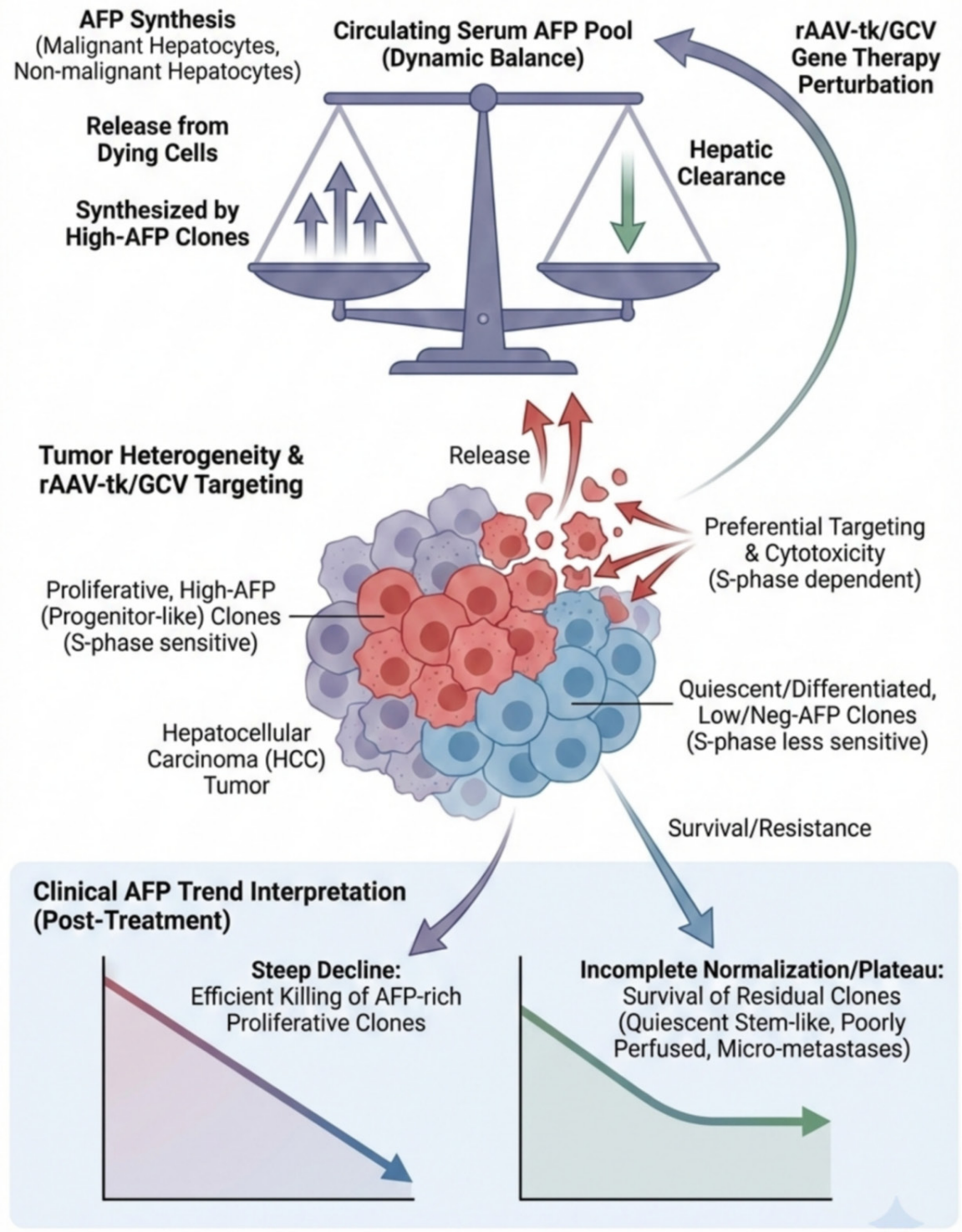

Figure 1. Mechanistic basis of serum AFP trajectories in rAAV-tk/GCV-treated hepatocellular carcinoma. Serum AFP reflects the net balance of production, release from dying cells, and hepatic clearance rather than a linear measure of tumor bulk. rAAV-tk/GCV preferentially eliminates proliferative, AFP-high tumor cells, producing steep early AFP declines, while sparing AFP-low/negative or quiescent subclones and poorly transduced regions that sustain residual AFP despite radiologic debulking. Recombinant adeno-associated virus-thymidine kinase/ganciclovir (rAAV-tk/GCV), Hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP).

tumor reduction therapy and relatively intact liver clearance function. However, in patients with advanced cirrhosis, a slow or delayed decrease in AFP is often due to limited liver clearance function and persistent regenerative AFP expression, rather than treatment failure [38, 39]. Thus, analyzing the change patterns of AFP-based biomarkers requires simultaneous assessment of liver function, fibrosis level, and time-varying indicators of hepatocyte damage and regeneration.

The innate and adaptive immune responses triggered by rAAV capsid, tk expression, and immunogenic tumor cell death provide an additional temporal regulatory effect on the later changes in AFP levels, which is particularly crucial [40, 41]. The cytotoxic T-cell effect against AAV capsid or tk-expressing cells may shorten the expression duration of tk, reduce the effective window of GCV dependent killing, and preferentially and selectively transfect tumor cell clones secreting AFP, ultimately forming a biphasic curve of AFP levels first decreasing and then increasing [42, 43]. Conversely, T cells can effectively cross-sensitize themselves to immunogenic cell death induced by endogenous tk/GCV, potentially triggering a secondary immune-mediated tumor control effect independent of direct tumor killing after the cytotoxic killing phase, thus maintaining AFP at a low plateau [44-46]. Based on the above multiple mechanisms, the AFP dynamics can be divided into stages and mechanistic definitions: first, the initial initiation/repression stage dominated by carrier biologic effects; second, the execution stage of tk/GCV cytotoxicity and bystander killing effect on tumor clones expressing high levels of AFP; and finally, the rebalancing stage under the combined effects of residual tumor biological characteristics, liver microenvironment, and immune dynamics. Transforming the mechanism of AFP time-varying curves into high-quality clinical applications is of great significance, since it can distinguish between kinetic patterns that indicate treatment resistance and those caused mainly by carrier heterogeneity, poor clearance function, or immune remodeling (**Figure 2**).

Application of AFP kinetics as a biomarker for efficacy assessment and prediction in translational applications

To translate AFP kinetics into a clinically valuable biomarker for in rAAV-tk/GCV-treated HCC, it is necessary to overcome fixed threshold limitations and define the data interpretation based on the mechanism of action. Unlike treating AFP as a binary variable (e.g., elevated vs. normal), its longitudinal changes can serve as a dynamic pharmacodynamic indicator of suicide gene activation and bodily response. Within the framework of translational research, early dynamic indicators such as the percentage decrease from baseline (e.g., a 50% or 75% decline), slope of logarithmically transformed AFP changes during the first treatment cycle, the time required to reach the lowest value, the level of the lowest value, and its duration are all potential early efficacy endpoints that may be correlated with radiographic tumor control, progression-free survival, and overall survival [47-50]. It is worth noting that, because rAAV-tk/GCV therapy exhibits selective cytotoxicity against proliferative tumor cells expressing high levels of AFP, a more significant early decrease in AFP levels can mechanistically correspond to a targeted tumor-killing effect. Conversely, a gradual decrease or early stabilization suggests poor vector transfection efficiency, insufficient GCV exposure, or residual lesions predominantly expressing low levels of AFP. Including pre-defined AFP kinetics parameters (similar to viral load kinetics in antiviral drug development) in Phase I/II clinical trials allows for more timely and rational decisions to continue or terminate the trial, and optimization of dosage and regimen, offering superior temporal resolution compared to traditional imaging examinations.

However, AFP kinetics must be used in combination with clinical scenarios and covariates at the patient and disease levels to become a reliable predictive biomarker. The morphology and reference value of the AFP curve depend upon baseline AFP levels (including absolute values and pre-treatment rate of increase), tumor burden and distribution, Child-Pugh liver function classification, underlying liver disease etiology, and the dosage and route of administration of the rAAV [51-53]. For patients with high baseline AFP levels and good liver function preservation, the percentage decrease in AFP and the rate of shortening of the half-life are likely to have high predictive value for sustained stable disease control. However, for patients with moderate baseline AFP levels, advanced cirrhosis, or both AFP-positive and AFP-negative lesions, the distinguishing effect of AFP changes of the

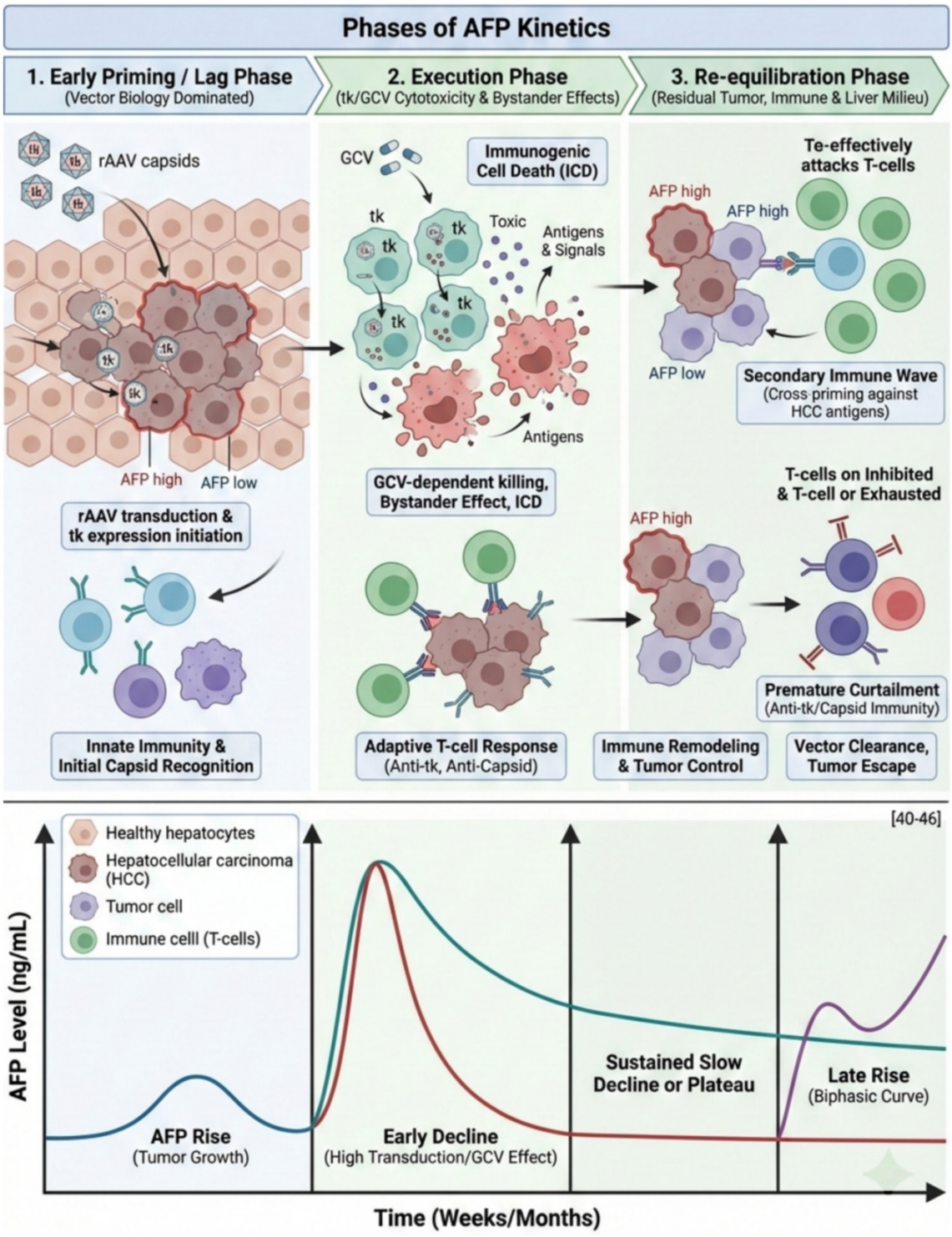

Figure 2. Immune-modulated phases of AFP kinetics after rAAV-tk/GCV in HCC. Innate and adaptive responses to rAAV capsid, tk expression, and immunogenic tumor cell death partition AFP kinetics into an early priming/lag phase, an execution phase driven by tk/GCV cytotoxicity and bystander effects in AFP-high clones, and a late re-equilibration phase shaped by residual tumor, liver milieu, and T-cell dynamics. Depending on whether anti-capsid/anti-tk responses prematurely terminate tk expression or effective cross-priming against HCC antigens is achieved, late AFP patterns diverge toward biphasic decline-rebound curves (tumor escape) or sustained slow decline/low-level plateaus (secondary immune-mediated control). Recombinant adeno-associated virus-thymidine kinase/ganciclovir (rAAV-tk/GCV), Hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP).

same magnitude may be poor. From a statistical perspective, combined models that integrate the longitudinal trajectory of AFP changes with time endpoint events (progression-free survival/overall survival), as well as analytical methods that combine AFP indicators with standard time points or response models, are more suitable for assessing the prognostic value of kinetic characteristics than univariate time point or response analyses. Mechanism-based parameters (such as biphasic decay patterns, representing rapid clearance of tumor cells expressing high AFP and slow changes in subsequent residual lesions) can be incorporated into such models to achieve individualized risk assessment and develop adaptive decision rules, such as identifying patients with poor AFP kinetics and enabling them to receive combination therapy or alternative regimens as early as possible.

All standardized operations that use AFP kinetics as a decision-making tool, as well as the handling of known biological and technical confounding factors, must be rigorous. At the technical level, it is necessary to unify the detection platform, calibration method and lower limit of quantification, and to sample according to a fixed geometric scheme and time arrangement (for example, testing weekly after each complete treatment cycle of GCV, and then every 4 to 8 weeks thereafter), so as to form a regular pattern for the detection of early inflection points and later rebalancing stages [54]. At the biological level, clinicians need to strictly distinguish the kinetic responses that are mechanistically independent: the short-term surge in AFP during GCV activation is consistent with cell necrosis and stress-induced release; while a sustained increase in the index is a typical feature of actual tumor progression; the appearance of a delayed plateau or decay trend is a typical manifestation of severe cirrhosis; a low-level plateau indicates low basic liver activity or the presence of minimal residual lesions. In practical applications, a composite response algorithm combining AFP kinetics with imaging examinations (such as volumetric or functional imaging), liver function tests, and, when conditions permit, circulating tumor DNA or other tumor-related biomarkers should be adopted, rather than relying solely on AFP indicators [55].

In future research, if it is fully integrated into mechanism-driven adaptive treatment strategies and biomarker-optimized trial designs, a complete translational application of AFP kinetics can be achieved in the rAAV-tk/GCV treatment system.

In the early development stages, kinetic characteristics based on molecularly anchored surrogate endpoints can be used for dose selection. They can also be used for comparing different serotypes, promoters, or GCV dosing regimens. In later stages of the trial, predefined AFP kinetic stratification (such as responders exhibiting late-stage slope decline and deep trough values) can guide patient enrichment, randomization, or regimen switching to maximize the therapeutic index. By combining each new test result and predicting dynamic changes in AFP using Bayesian methods or models, dynamic interventions can be implemented at the individual patient level, including increasing GCV dosage, adopting local or systemic therapy, or discontinuing ineffective treatment for non-responders. Finally, for AFP kinetics to become a high-quality biomarker in rAAV-tk/GCV therapy, prospective multicenter validation, external reproduction of the kinetic-response-prognostic association, and collaboration with regulatory agencies to establish acceptable kinetic endpoints are necessary. Implementing these measures will transform AFP from a traditional tumor marker into a pharmacodynamic tool that can be interpreted mechanistically and analyzed quantitatively, significantly promoting the development of suicide-gene therapy and use in HCC (**Figure 3**).

Implications for future research and clinical practice

This review reveals that serum AFP kinetics can serve as a biomarker with rich mechanistic implications in rAAV-tk/GCV therapy for HCC. This conclusion has significant value for future research directions. First, prospective studies are urgently needed in this field to specifically study AFP as a dynamic biomarker, rather than treating it merely as a secondary laboratory indicator. Future clinical trials should pre-plan high-resolution longitudinal AFP testing, rather than using AFP as an intermittent auxiliary test for imaging examinations [56]. Intensive sampling during the early initiation and implemen-

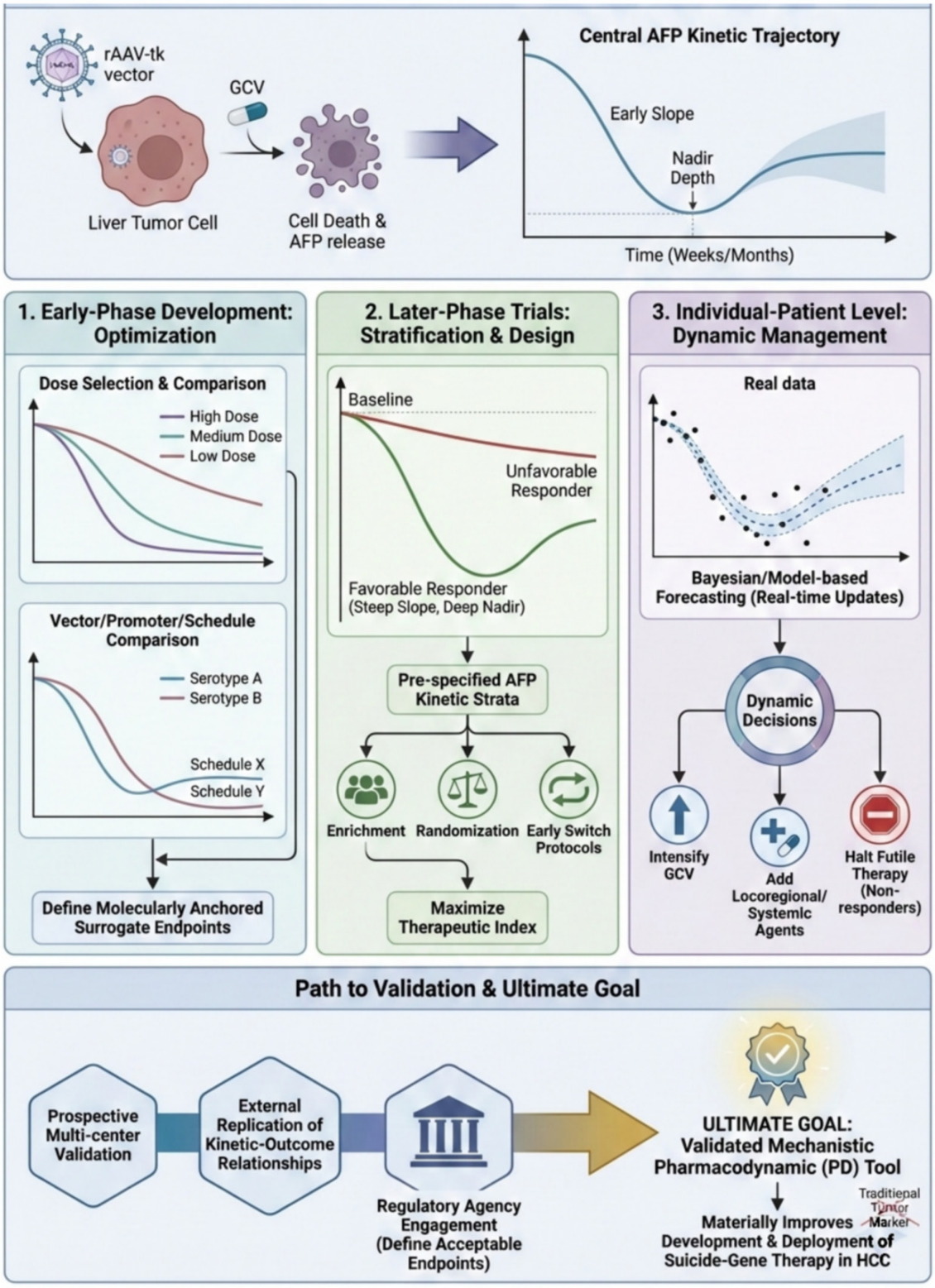

Figure 3. Translational roadmap for using AFP kinetics as a mechanistic pharmacodynamic biomarker in rAAV-tk/GCV-treated HCC. Central AFP trajectories (early slope and nadir depth) are leveraged as mechanistic PD readouts to optimize vector dose and schedule in early-phase studies, to predefine kinetic strata for enrichment and early-switch designs in later-phase trials, and to enable Bayesian, patient-level forecasting that guides dynamic decisions (intensifying GCV, adding locoregional/systemic agents, or stopping futile therapy). Prospective multicenter validation and regulatory qualification could ultimately establish AFP kinetics as a top-tier biomarker for suicide-gene therapy in HCC. Recombinant adeno-associated virus-thymidine kinase/ganciclovir (rAAV-tk/GCV), Hepatocellular carcinoma (HCC), alpha-fetoprotein (AFP).

tation phases of treatment and continuous monitoring during the rebalancing phase are crucial to identify reproducible kinetic prototypes and establish their correlation with molecular, immune, and imaging-related indicators. Therefore, future trials should incorporate pre-defined kinetic analyses, including the construction of multi-stage decline models, estimation of AFP half-life, quantitative analysis of transient spikes and plateau phases, and joint modeling strategies that correlate AFP change trajectories with disease progression and survival endpoints. Equally important, it is necessary to verify the mechanistic hypotheses behind specific kinetic patterns through a biomarker research protocol that integrates imaging, liver function indicators, immune monitoring, and continuous carrier detection. For example, whether an early spike in AFP reflects cell necrosis, inflammatory release, or biological resistance, and whether a later plateau phase represents impaired clearance function or minimal residual disease.

The second major direction is to use AFP kinetics as a feedback tool to optimize the design of rAAV-tk/GCV-based therapy. Future research must use AFP time-varying curves as feedback indicators to assist in the optimization of vector design, dosage, and combination therapy regimens [57, 58]. In this context, AFP kinetics should be regarded not only as an outcome evaluation indicator, but also as a real-time biomarker reflecting biological effects to guide the optimization of treatment regimens. For example, the inconsistent phenomenon of early decline but later rebound of AFP may indicate incomplete intratumoral transfection coverage, insufficient bystander killing effect, or inability to control drug-resistant subclones with low AFP expression, thus providing a basis for research directions such as the construction of dual promoter vectors, alternative capsid serotypes with better tumor penetration, and combination of local or systemic drugs targeting resting or stem cell-like tumor cells [59]. Similarly, a slight decrease in AFP levels in the presence of high-titer neutralizing anti-AAV antibodies or strong anti-capsid cell immunity helps identify patients requiring immunogenicity reduction strategies or adjusted dosing regimens [60-62]. Besides specific rAAV-tk/GCV systems, the above concepts may also provide a reference for other gene therapy and tumor-targeted immunotherapy regimens. In these treatments, AFP kinetics can serve as a universal pharmacodynamic evaluation indicator, facilitating cross-platform comparisons of biological activity, which traditional imaging efficacy evaluation standards cannot fully reflect [63].

The third research focus is on constructing a composite biomarker system, because relying solely on AFP kinetics is insufficient in all biological or clinical scenarios. The liver's baseline microenvironment, differences in liver function reserve, and the presence of low AFP or AFP-negative tumors all significantly affect the interpretation of AFP results, reducing the reliability of a single kinetic signal. Future research needs to establish a composite kinetic profile of AFP and other serum biomarkers (such as AFP isoform L3, abnormal prothrombin, or indicators related to inflammation and liver regeneration), circulating tumor DNA, and quantitative imaging methods. In low-AFP or AFP-negative HCC, other analytes are particularly important, as biomarkers with similar mechanisms can more accurately reflect the kinetics of protein secretion, release, and clearance [64-66]. Simultaneously, constructing large-scale, high-quality labeled datasets that include longitudinal biomarker detection data, imaging results, tumor genomic characteristics, immune status, liver function, and clinical outcomes is crucial for clarifying when AFP kinetics can independently provide effective information and when it needs to be interpreted in conjunction with multimodal indicators [67, 68]. Related research also needs to consider etiological heterogeneity, including viral and non-viral HCC, different stages of cirrhosis, and significant differences in the accessibility of detection methods, sampling frequency, and follow-up systems in different clinical settings worldwide.

Finally, to apply the above research findings to clinical practice, AFP kinetics needs to be transformed into practical and standardized clinical tools. Future research should not rely solely on fixed thresholds, but rather translate AFP kinase levels into actionable clinical decision-making rules. This would help clinicians distinguish between treatment-related benign fluctuations and actual disease progression, identify characteristic patterns indicating insufficient carrier activity or impaired liver clearance, and provide a scientific basis for continu-

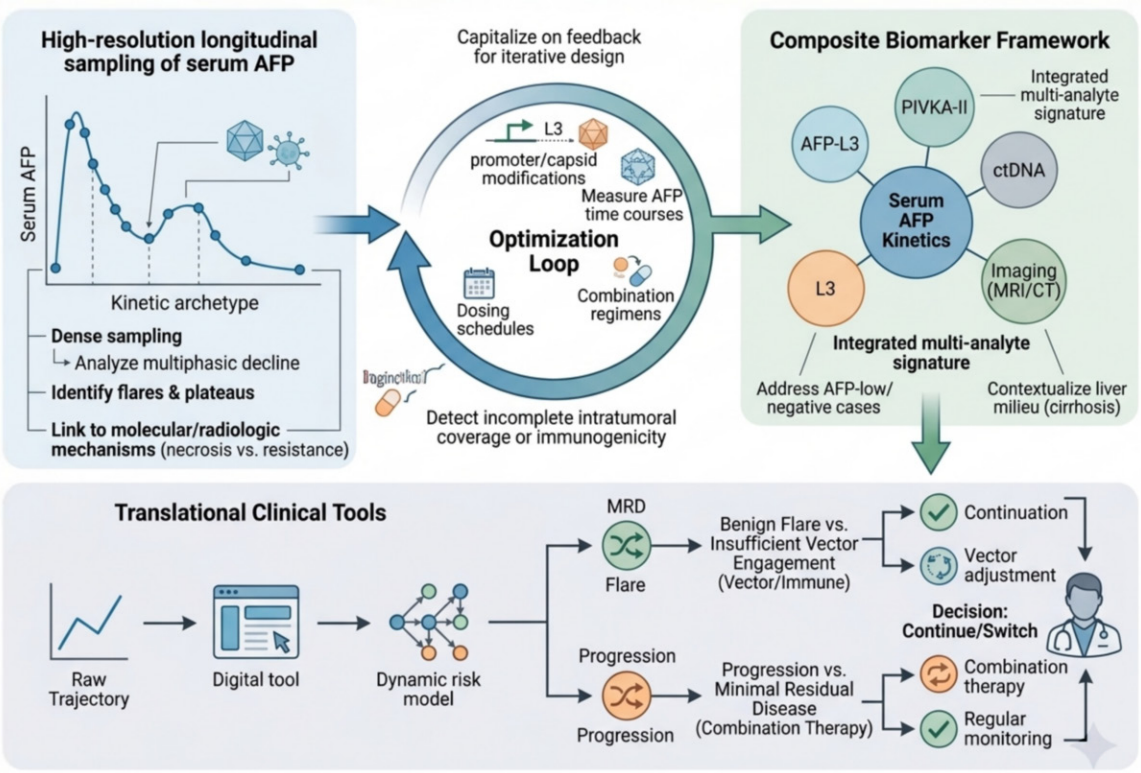

Figure 4. Translational roadmap for establishing serum AFP kinetics as a mechanistically informed pharmacodynamic biomarker in rAAV-tk/GCV-treated hepatocellular carcinoma. This schematic summarizes a forward-looking framework for the clinical and translational deployment of serum AFP kinetics in HCC treated with rAAV-tk/GCV suicide gene therapy. The roadmap begins with high-resolution longitudinal AFP sampling, enabling identification of reproducible kinetic archetypes, including multiphasic decline, transient flares, and plateau patterns, and linking these trajectories to underlying molecular, radiologic, and immunologic processes. AFP time-course data are then incorporated into an optimization loop to refine vector design, promoter/capsid selection, dosing schedules, and combination regimens, while also helping detect incomplete intratumoral coverage or treatment-limiting immunogenicity. At the biomarker level, AFP kinetics is positioned within a composite framework that integrates complementary analytes, including AFP-L3, PIVKA-II, circulating tumor DNA, and quantitative imaging, thereby improving biological interpretation, especially in AFP-low or AFP-negative disease and within the context of cirrhosis. Finally, these integrated kinetic signals are translated into practical clinical tools, including digital platforms, dynamic risk models, and decision algorithms that distinguish benign AFP flares from minimal residual disease, progression, or insufficient vector engagement, and thereby support individualized decisions regarding treatment continuation, vector adjustment, combination therapy, or routine monitoring. Collectively, this framework highlights how mechanistically interpreted AFP kinetics may evolve from a conventional tumor marker into a clinically actionable biomarker for response assessment, therapeutic optimization, and precision management of rAAV-tk/GCV-based therapy in HCC.

ing, intensifying, or changing treatment regimens. This requires developing validated nomograms, dynamic risk models, and convenient digital decision support systems that integrate AFP kinase levels, baseline clinical indicators, liver function data, and imaging results. Professional societies and expert alliances also need to develop standardized sampling protocols, reporting formats, and efficacy evaluation criteria, explicitly incorporating kinetic measurements and achieving standardization across clinical centers and clinical trials. In the long term, for AFP kinetic endpoints to gain regulatory approval and become alternative or adjunctive biomarkers in rAAV-tk/GCV and related treatment systems, rigorous prospective multicenter validation and close integration of mechanistic studies and clinical evidence are essential. With systematic advancement of related research, AFP has the potential to evolve from a traditional tumor marker into a core indicator with mechanistic interpretation value and clinical guidance significance in the precise monitoring of HCC gene therapy (**Figure 4**).

Conclusion

Existing data and mechanistic analyses support a unified view. In HCC treated with rAAV-tk/GCV, serum AFP kinetics is a high-value but currently underutilized biomarker bridging biological mechanisms and clinical practice. rAAV-tk/GCV suicide gene therapy interferes with tumor burden, clonal composition, liver regeneration, and anti-tumor immune status in AFP-secreting tumors, and this interference is comprehensively reflected through time-dependent changes in circulating AFP. Redefining AFP as a dynamic pharmacodynamic response indicator rather than a simple diagnostic biomarker can uncover early evidence of efficacy or resistance, construct core efficacy and resistance driver models, and provide rational insights for vector design, dosage, and combination regimen formulation. To put this concept into practice, intensive and standardized *in vivo* AFP testing is needed, along with combined analysis of its kinetic indicators with imaging, liver function, and immune biomarkers, and advanced longitudinal data analysis methods to extract reliable and clinically significant kinetic profiles. Ultimately, after rigorous validation across different etiologies, disease stages, and clinical scenarios, AFP kinase dynamics can serve not only as a classic tumor marker but also as a quantifiable and mechanistic-guided decision support tool. This tool can be applied to rAAV-tk/GCV gene therapy to accelerate research and development process, optimize efficacy evaluation, and allow clinical treatment of HCC guided by biomarkers.

Disclosure of conflict of interest

None.

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