Advancements in Chimeric Antigen Receptor T Cell Therapy for Hepatocellular Carcinoma

*Xinyu Zhao**
School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Abstract Chimeric antigen receptor T cell (CAR-T) therapy nowadays symbolizes an innovative paradigm of neoplasm immunotherapy that employs genetic engineering techniques to empower T cells and make it have a capability to precisely identify tumor-associated antigens, thereby unleashing potent anti-tumor responses. The remarkable success observed in leukemia and lymphoma treatment has shown the possibility for extending CAR-T therapy's potential to solid tumors. In the context of hepatocellular carcinoma (HCC), the prevailing form of primary hepatic malignancy, diagnosis frequently occurs during advanced stages, imposing significant challenges. The current options available for managing HCC are notably limited, and though previous research has showcased the feasibility of CAR-T cell application, achieving optimal therapeutic outcomes has remained elusive. This phenomenon can be induced by various factors including adverse effects， tumor microenvironment, the diversity inherent in tumor antigens, heightened intratumor pressures and the immunosuppressive context within exhaustion of CAR-T Cells. All of these reasons undermine the effectiveness of this therapy and a multitude of ongoing clinical investigations and preclinical research are underway to overcome these hurdles. This comprehensive review concisely outlines the function of this therapy, existing targets of HCC treatment and examines the current impediments and offers potential resolutions within the context of HCC.

1. Introduction

Among all malignant tumors worldwide, liver cancer emerges as a prominent adversary, ranking sixth in terms of its widespread incidence, registering a staggering count of over 905,000 novel cases during the year 2020. Furthermore, its presence is acutely felt within the spectrum of mortality, contributing to a substantial toll of approximately 83,500 lives lost [1]. Foremost among the constellation of liver cancers is hepatocellular carcinoma (HCC), encompassing a formidable share of 75-85% among primary hepatic malignancies. The genesis of HCC is deeply rooted in an intricate interplay of multifarious instigators. These encompass a convergence of factors ranging from viral intruders, exemplified by the hepatitis B virus and C virus, to the repercussions of alcohol consumption, aflatoxin exposure, the metabolic intricacies of diabetes, and the labyrinthine realm of metabolic syndrome. Notably, the dominion of HCC's influence is disproportionately felt across Sub-Saharan Africa and Asia, regions where the relentless grip of chronic HBV infection perpetuates its reign with a tenacious vigor. Approximately 40% of HCC cases on a global scale bear the hallmarks of this unyielding chronic HBV association [2]. However, intriguing shifts have come to light, unveiling an unsettling surge in HCC incidence in domains that have traditionally remained categorized as low-risk. Western nations, previously sheltered from this onslaught, now grapple with this rising tide, attributed in part to the insidious expansion of obesity and non-alcoholic fatty liver disease (NAFLD).

Tumor immunotherapy has garnered significant attention due to its impressive ability to induce curative responses. As of March 2019, the global biomedical landscape has witnessed the emergence of an astounding 1011 cellular therapy products. Notably, therapeutic agents derived from CAR-T cell therapy have taken center stage, boasting an impressive count of 568, a notable achievement that far surpasses alternative therapeutic modalities [3]. As a genetic engineering, this therapy genetically manipulates T lymphocytes to proficiently encode chimeric antigen receptors, enabling precise and enhanced antigen recognition and immune response activation. These receptors empower the modified T cells to distinctly identify and interact with antigens which are expressed on the surface of malignant cells, thereby initiating their targeted elimination. CAR-T therapy initially demonstrated its efficacy in addressing hematological malignancies, yielding promising outcomes. Two discrete CAR-T cell therapeutics (Yescarta and

* Corresponding author's email: 1911441109@mail.sit.edu.cn

Kymriah) engineered to confront acute lymphoblastic leukemia and recalcitrant or relapsed large B-cell lymphoma in a discrete manner, have been authorized by the US Food and Drug Administration (FDA). Scholars have also undertaken investigations into the potential utility of this treatment in tackling solid malignancies. To date, numerous academic institutions have adeptly executed comprehensive foundational and clinical investigations encompassing CAR-T therapy's utility in addressing solid malignancies such as glioblastoma, melanoma, ovarian carcinoma, and hepatocellular carcinoma (HCC) [4]. This review focuses on discussing the specific targets and the latest progress made in treating HCC by using this promising immunotherapy.

2. Generation and functionality of CAR-T cells

At present, T lymphocytes can be genetically modified through the utilization of viral or non-viral vectors harboring the chimeric antigen receptor (CAR) construct. Viral vectors exhibit heightened proficiency in gene transfer, expediting the amplification of T cells to achieve the requisite therapeutic dosage within a comparatively truncated timeframe. Furthermore, distinct patterns of CAR expression are observed among diverse viral vectors, affording a spectrum of options for foundational scientific inquiry and clinical experimentation [5]. Amid viral vectors, retroviral and lentiviral vectors predominate in application, although latent health hazards persist, encompassing potential immune reactivity, toxicological ramifications, insertional mutagenesis, or instigation of tumorigenic propensities. Conversely, non-viral vectors hold sway by virtue of their non-infectious nature, facile scalability, expanded vector carrying capacity, and manageable molecular architecture, thus drawing heightened scholarly interest. Transposon-based systems, inclusive of the predominant Sleeping Beauty, PiggyBac, and Tol2 transposon systems, collectively comprise the principal category of non-viral vectors. Notably, the realm of RNA-guided electroporation in lymphocytes has emerged as a secure and economically viable avenue, albeit wielding inferior efficiency in contrast to the lentiviral approach. Consequently, a judicious assessment of the idiosyncrasies underpinning these disparate methodologies is requisite to discern the optimal approach for engineering CAR-enhanced T cell populations.

The therapeutic prowess of CAR-T cells stems from their multifaceted mechanisms of action. Once infused into the patient, CAR-T cells recognize TAAs expressed on cancer cells' surface. This recognition triggers a cascade of events, including T cell activation, cytokine release, and cytotoxic granule secretion. The cytotoxic effectors, such as perforin and granzymes, mediate the elimination of cancer cells. Notably, CAR-T cells exhibit persistence in the body, enabling them to mount sustained antitumor responses. Additionally, CAR-T cells can induce immunological memory, conferring long-term protection against disease recurrence. When compared with traditional immunotherapies, CAR-T cell therapy showcases distinct advantages. Unlike checkpoint inhibitors that target immune suppression, CAR-T cells make the immune system powerfully eradicate cancer cells. Moreover, CAR-T cells exhibit remarkable efficacy even in refractory or relapsed cases, where conventional treatments falter. This therapy also demonstrates potential in treating hematological malignancies and solid tumors. However, it's important to note that it can elicit severe adverse effects, including cytokine release syndrome and neurotoxicity. Contrasting adoptive T cell transfer and immunotherapeutic vaccines, CAR T cells exhibit higher specificity due to their precisely engineered CARs. In contrast, adoptive T cell transfer relies on endogenous T cells, which may lack specificity and require ex vivo manipulation. Therapeutic vaccines, while stimulating the immune system, might not achieve similar level of targeted cytotoxicity like CAR-T cells [6]. Thus, this therapy stands as a remarkable advancement in personalized medicine, harnessing the potential of genetic engineering for targeted and potent antitumor responses.

3. Targets aimed by CAR-T cell therapy for hepatocellular carcinoma

3.1 Glypican-3 (GPC3)

GPC3, a heparan sulfate proteoglycan consisting of 580 amino acids, emerges as an overexpressed entity in numerous malignancies, hepatocellular carcinom, melanoma , ovarian carcinomas, and lung squamous cell carcinoma among them. However, its presence in normal tissues, including normal liver tissue and liver cirrhosis, is either absent or marked by very low expression. A study by Capurro et al. unveiled that 72% of patients suffering from HCC have this antigen, with serum GPC3 levels notably elevated in 53% of these patients [7]. Wnt signaling pathway is regared to facilitate the progress of hepatocellular carcinoma(HCC). GPC3 has attributed function involves fostering HCC development by activating this pathway. Another research evidenced that silencing GPC3 on HCC cells impaired their proliferative and invasive potential, underscoring the involvement of GPC3 in these processes. This finding comes from Morgan et al. Moreover, GPC3 overexpression also serves as a harbinger of unfavorable prognosis. Consequently, GPC3's robust specificity and heightened sensitivity position it as a pivotal target in both HCC diagnosis and treatment.

Pioneering the endeavor, the first GPC3-specific CAR-T cells was made by Gao et al., which was engineered to target glypican-3 (GPC). A comparative assessment between this CAR-T cells of the first and third generations exhibited that they proficiently inhibited the proliferation of hepatocellular carcinoma

(HCC) cells both under laboratory-controlled in vitro conditions and within living organisms in vivo. Notably, the CAR-T cells of the third evolutionary iteration exhibited superior efficacy compared with their first-generation counterparts. A separate research investigation delineated the utilization of three hepatocellular carcinoma (HCC) models originating from patient-derived xenografts (PDX) that feature GPC3 expression. This study illustrated that GPC3-targeted CAR-T cells impeded tumor progression, albeit exhibiting varying degrees of effectiveness due to the heterogeneous distribution of programmed death-ligand 1 (PDL1) expression on the malignant cell population [8]. This finding postulates the feasibility of synergizing CAR-T therapy with immune checkpoint inhibitors to attain heightened efficiency in eradicating PD-L1-positive HCC. Significantly, a multitude of ongoing clinical trials are presently scrutinizing GPC3-targeting CAR-T therapy's potential for the management of advanced hepatocellular carcinoma. Noteworthy among these trials are two studies (NCT02395250, NCT03146234) which have successfully completed the enrollment phase..

3.2 Alpha-fetoprotein (AFP)

Alpha-fetoprotein (AFP) is an extracellular glycoprotein secreted into the systemic circulation. This glycoprotein consists of a sequence of 591 amino acids and encompasses approximately 4% of its composition as carbohydrate residues. Its molecular mass is estimated to be approximately 70 kilodaltons (kDa). Notably, AFP serves various vital physiological roles, including transport, immunosuppression, and apoptosis induction [9]. While the fetal serum showcases high levels of AFP, its presence diminishes significantly in adulthood. However, when conditions such as teratoma, hepatoblastoma, and hepatocellular carcinoma arise in individuals of mature age, AFP expression resurfaces. This revival enables its utility as a circulating biomarker for diagnosing tumors and surveillance of treatment efficacy.

Elevated levels of AFP are evident in the serum and tumor tissues of 60% to 80% of liver cancer patients, making it an potential aim . CAR-AFP-T cells were developed using AFP as the target antigen and validated in a Hep G2 xenograft mouse model. Results revealed that, following intravenous administration, approximately 17% of mice exhibited significantly reduced tumor volumes compared to the control group. With intratumoral injection, about 75% of mice experienced complete regression of tumors, accompanied by no residual tumor tissue.

Conventional CAR-T cells are exclusively designed to engage cell surface markers found on tumor cells, omitting intracellular or secreted antigens from their scope. However, it is worth noting that intracellular or secreted proteins undergo processing to generate peptides, subsequently displayed by class I major histocompatibility complex (MHC) molecules at the surface of tumor cells. In the work by Liu et al., they designed AFP-specific CAR-T cells which demonstrate a distinct affinity for the AFP158–166 peptide, presented by HLA-A02:01. This recognition event leads to degranulation, cytokine release, and the subsequent lysis of HLA-A02:01+/AFP+ tumor cells [10]. Furthermore, These cells have demonstrated their efficacy in hindering tumor growth in xenograft models and have progressed to Phase I clinical trials (NCT03349255), effectively assessing the security as well as therapeutic efficacy in patients whose cancer cell expressing AFP. Consequently, the strategic targeting of intracellular tumor antigen products by CAR-T therapy holds great significance in HCC. Besides,targeting secreted antigen also presents substantial potential for advancing cancer treatment modalities.

3.3 Epithelial cell adhesion molecule (EpCAM)

EpCAM, a transmembrane glycoprotein, showcases notable levels of expression across a spectrum of human malignancies that originate from epithelial tissues, including hepatocellular carcinoma (HCC). Previous studies have elucidated that EpCAM-positive (EpCAM(+)) HCC cells possess traits reminiscent of hepatic stem cells, such as the ability to undergo self-renewal and differentiate into various lineages, which fundamentally influence HCC's growth and invasiveness . Furthermore, Yamashita et al. scrutinized EpCAM's signature across more than 150 tumor samples and substantiated that HCC patients presenting both EpCAM and AFP exhibited prolonged survival and a higher occurrence of portal vein invasion compared to those without EpCAM and AFP expression [11]. As a result, EpCAM has emerged as both a prospective therapeutic target and an incipient-stage biomarker for hepatocellular carcinoma (HCC).

At present, CAR-T cells engineered to specifically target EpCAM have been engineered and tested for therapeutic purposes in colorectal cancer (34). Nevertheless, their potential relevance in hepatocellular carcinoma (HCC) has not yet been examined within the preclinical domain. A phase I/II clinical trial (NCT03013712) is presently in progress to assess the therapeutic effectiveness of EpCAM-targeting CAR-T cells against cancers that exhibit EpCAM expression. Concurrently, an alternative trial is in the process of enlisting 25 participants diagnosed with advanced HCC, recurrence after surgical intervention, or resistant HCC to systematically assess the security characteristics of CAR-T cells directed against EpCAM (NCT02729493).

3.4 CD133

CD133 stands as a pentaspan transmembrane glycoprotein, marked by elevated expression in hepatocellular carcinoma (HCC), pancreatic cancers and other solid tumors . Moreover, CD133 assumes a prominent role as a discernible biomarker indicative of cancer stem cells (CSCs), critical entities with pivotal control over tumor viability, proliferation, metastatic

potential, and relapse propensity. In their study, Kohga et al. documented the selective occurrence of CD133-positive cells solely within hepatocellular carcinoma (HCC) tissues, while they were notably absent in non-neoplastic hepatic tissues. Their findings additionally suggested that the upregulation of CD133 expression within hepatocellular carcinoma (HCC) cells could potentially instigate the augmentation of cellular proliferation and the facilitation of metastatic processes [12]. Prior investigations have showcased a correlation between heightened CD133 levels in HCC patients and diminished rates of overall survival coupled with escalated frequencies of disease recurrence. Hence, CD133 emerges as a potential molecular target for immunotherapeutic interventions in patients diagnosed with hepatocellular carcinoma (HCC) exhibiting CD133-positive tumor profiles.

In a pioneering investigation, Wang et al. created CD133-selective CAR-engineered T-cells (CAR-T-133) , thus uncovering their remarkable proficiency in lysing CD133+ cells as well as generating elevated cytokine levels against them. These CAR-T-133 cells notably inhibited tumor growth in vivo, illustrating an elevated abundance of CAR genetic element copies within the tumor tissue in contrast to the comparator cohorts. Subsequently, they initiated a prospective clinical investigation or clinical study (NCT02541370) to systematically assess the tumor-suppressing effectiveness of CAR-T cells against CD133 in individuals afflicted with serious hepatocellular carcinoma (HCC). Within this investigation involving a cohort of 14 participants, a notable 64% achieved a state of stable disease, correlating with a 43% disease control rate sustained over a span of 6 months. The application of immunohistochemistry shed light on the post-administration abatement of CD133+ cells following the infusion of CAR-T-133. The recorded median timeframe of progression-free survival as witnessed in patients afflicted with hepatocellular carcinoma subjected to this therapy (7 months) demonstrated a substantial increase in contrast to the progression-free survival duration observed in patients with advanced HCC unresponsive to primary-line treatments and sorafenib (4 months). Furthermore, the implementation of repeated CART-133 cell therapy showcased a favorable clinical response among HCC patients carrying a diminished tumor burden or presenting with early-stage tumors.

CD147, classified as a type I transmembrane glycoprotein, has attracted significant interest owing to its pronounced presence in approximately 80% of liver malignancies and other neoplasms, encompassing pulmonary malignancies, breast carcinoma and urinary bladder cancer . Extensive research elucidates CD147's contribution to tumor progression, invasion, and metastasis through its capacity to stimulate the secretion of MMPs (matrix metalloproteinases) [13]. Moreover, recent studies have drawn a connection between CD147 expression and aggressive behavior as well as poor prognosis in HCC . Recent strides in the field have led to the emergence of an innovative 131I-labeled monoclonal antibody specifically designed to target CD147. This groundbreaking approach has been employed with radiofrequency ablation (RFA) in synergy. Additionally, transcatheter arterial chemoembolization (TACE) is also harnessed with this method for the management of hepatocellular carcinoma (HCC) . These revelations collectively accentuate the viability of targeted therapeutic interventions centered on CD147 as a compelling avenue for effectively addressing HCC.

Examining the clinical response of CD147-targeting CAR-T cells, a Phase I clinical investigation (NCT03993743) was conducted, enrolling individuals diagnosed with highly progressed hepatocellular carcinoma (HCC). Notably, an innovative methodology was embraced, characterized by the utilization of a genetically engineered CAR-T cells with inducible activation governed by the Tet-On regulatory network. This unique construct enabled the reversible activation or deactivation of the CAR gene's expression contingent upon the presence or absence of doxycycline (Dox). Of paramount importance, the initiation of Dox administration could be promptly ceased upon the occurrence of severe adverse reactions, resulting in the swift reversal of CD147 CAR expression levels on T lymphocytes to their original state within a temporal range of 24 to 48 hours. In vitro experimentation unveiled that (Dox+) Tet-CD147CAR-T cells exhibited enhanced cytotoxic potential and elevated cytokine secretion in comparison to (Dox−) Tet-CD147CAR-T cells and peripheral blood mononuclear cells (PBMCs). Furthermore, (Dox+) Tet-CD147CAR-T cells effectively arrested the proliferation of tumor cells within the xenograft model of hepatocellular carcinoma (HCC). These findings illustrate that Tet-CD147 CAR-T cells have the potential to be modulated through Dox administration, evident in both laboratory-based in vitro assessments and live organism-based in vivo evaluations. This regulatory mechanism holds promise in attenuating the potential toxicity and untoward repercussions.

4. Adverse Effects of CAR-T Cell Therapy

Within the implementation of CAR-T cells in clinical medicine, the most commonly encountered adverse events encompass off-target effects, frequently denoted as “on-target, off-tumor” toxicities. The occurrences of this side effect cause an autoimmunological reaction directed targeting non-pathological tissues expressing the intended antigen target. This challenge predominantly emerges within therapeutic paradigms focused on solid tumors. A preceding clinical trial unveiled instances of hepatotoxicity in subjects infused with self-derived T lymphocytes that have been modified genetically to incorporate a chimeric antigen receptor (CAR) specifically designed to recognize carbonic anhydrase IX (CAIX). The immunogenic detection of carbonic anhydrase IX (CAIX), which is also present in normal bile duct epithelium, was ascribed to this hepatotoxic response [14].

Nevertheless, subsequent investigations illustrated that this on-target toxicity will be circumvented by administering first-generation CAR constructs in tandem with pre-treatment using a monoclonal antibody (mAb) targeting CAIX, at the minimal effective T cell dosage.

Furthermore, the on-target off-tumor effect was linked to a life-threatening case of toxicity. Some tissues like heart could be inadvertently targeted by the anti-HER2 CAR-T cells, wherein trace ERBB2/HER2 levels were present. This led to a cytokine storm and subsequent respiratory system-related adverse effects .

Moreover, instances of neurological toxicity happened in experiments characterized by elevated serum cytokine levels. These neurological complications were marked by symptoms including headaches, cognitive disarray, perceptual distortions, speech impairment, motor planning difficulties, lack of coordination, and convulsive episodes. While the elevated quantities of interleukin-2 (IL-2) has been associated with generalized encephalopathy in patients who suffer solid tumors, how neurological toxicity would occur and its precise mechanism remains inadequately comprehended.

Another substantial risk to the medical security of this therapy arises from cytokine release syndrome (CRS), commonly initiated when a substantial number of potent CAR-T cells are introduced into the organism. A complication with the potential to jeopardize patient survival(CRS), entails the liberation of proinflammatory cytokines such as IL6 from immune system cells into the circulatory system. This release precipitates symptoms like high fever, hypotension, and organ dysfunction. Both neoplastic load and the quantity of infused CAR-T cells are highly connected to the intensity of CRS. Notably, CRS tends to be more prevalent in trials focused on hematological malignancies compared to those involving solid tumors, because of the inherent complexities CAR-T cells face when targeting solid tumors [15]. Notably, CRS and neurological toxicity often co-occur within the same patient groups, suggesting a potential interconnectedness between these two conditions.

Additionally, tumor lysis syndrome (TLS), another potentially lethal complication, may result from the abrupt and extensive discharge of biologically active molecules subsequent to the swift disintegration of tumor cells. This phenomenon transports intracellular molecules to the extracellular milieu, thereby possibly disrupting physical microenvironment and perturbing physiological homeostasis. Moreover, the incorporation of retroviral or lentiviral vectors—employed for the integration of the chimeric antigen receptor (CAR) genetic construct into T lymphocytes poses a prospective security hazard, as observed in gene therapy investigations pertaining to primary immunodeficiencies.

5. Enhancing the safety profile of CAR-T cell therapy

With the aim to optimize the therapeutic effectiveness of CAR-T cell therapy while concurrently mitigating undesirable adverse events, the enhancement of CAR-T cell manufacturing methodologies assumes pivotal significance. The importance of this therapy lies the crucial necessity to discriminate between non-neoplastic and neoplastic cells. Presently, only a limited subset of CARs exhibit robust tumor specificity. Thus, the judicious selection of appropriate target antigens, coupled with the enhancement of tumor selectivity, is imperative to circumvent off-target repercussions. Addressing the constraints of single-tumor antigen specificity, the integration of dual CAR targeting technology emerges as an alternate approach. This process involves the genetic engineering of T lymphocytes constructed with a pair of discrete chimeric antigen receptors (CARs): one comprising a CD3ζ activation domain and the other integrating a co-stimulatory activation domain. The activation of dual CAR-T cells necessitates concurrent engagement of both CAR antigens at the surface of cancer cells. In situations involving non-neoplastic cells, the initiation of antigenic activation signals is feeble, leading to the non-engagement of co-stimulatory signals. As a result, the activation cues for T cells remain inadequate, precluding any detrimental impact on normal cellular constituents [16].

Furthermore, manipulating the affinity of CAR-T cells presents a propitious avenue for exploration. Empirical evidence underscores that CARs with lower-affinity single-chain variable fragment (scFv) recognition augment specificity towards tumor targets that are overexpressed compared to their physiological levels in healthy tissues. Antigen-specific inhibitory CAR (iCAR) technology offers another strategic pathway [17]. Integrating an "inhibitory brake" within highly active CAR-T cells, exerting restraint through intracellular PD-1 or CTLA-4 domains, curtails T cell activation signals emanating from the CAR. This orchestrated deactivation forestalls the genetically modified T cells from engaging normal cells, averting on-target off-tumor outcomes and, in specific contexts, mitigating cytokine release syndrome (CRS).

To alleviate side effects, incorporating a "suicide gene" into transduced cells is a prevalent approach. These gene products elicit cytotoxicity, independent of external agents, and inhibit antigen receptor expression. This elicits the targeted elimination of genetically modified cells, preemptively mitigating prospective CAR-T cell-associated toxicities. Despite its efficacy, this approach presents a dual-sided proposition. For instance, the incorporation of the herpes simplex virus thymidine kinase (HSV-TK) gene could elicit immune reactions, whereas the manipulation of the inducible caspase 9 (iCasp9) gene initiates programmed cell death within the transferred T cells [18].

Contrasting stable CAR-T cell engineering, transient CAR expression can be induced via T cell transfection with RNA. Cytokine receptor blockade can alleviate CRS. By judiciously modulating transfection doses, this approach offers potential mitigation of CAR-associated toxicity. However, the

trade-off involves a potential compromise in the therapeutic potency.

In summary, enhancing CAR-T therapy effectively resides in minimizing grave complications while ensuring therapeutic efficacy. The meticulous balance between potency and safety remains the ultimate objective, steering the ongoing evolution of CAR-T therapy toward ever-improving patient outcomes.

6. Conclusion

Nowadays, CAR-T cell therapy has drawn more attention in targeting and treating hematological malignancies. However, there remains a considerable distance to reach a dependable remedy for solid tumors, notably Hepatocellular Carcinoma (HCC). As deliberated upon within this comprehensive assessment, several encouraging strategies have been conducted within preclinical models, yielding auspicious findings in CAR-T cell therapeutics for HCC. Moreover, with the emergence of engineering affinity-adjusted CAR-T cells, more novel avenues for circumventing undesirable side effect, such as on-target off-tumor toxicities, has been invented. Besides, improved CAR-T cell therapy containing chemokine receptor manipulation presents an enticing prospect, streamlining T cell infiltration and fostering enduring presence. In addition, many distinctive therapeutic paradigms geared towards suppression or mitigation of immunosuppressive cell populations and inflammatory mediators have been proffered due to the immunosuppressive microenvironment. In pursuit of augmented safety and efficacy, some methods including switch-based CAR-T cells emerges as the effective and propitious therapeutic modalities in treating various malignancies. To encapsulate, genetically engineered CAR-T cells stand as an exceptionally promising frontier in the domain of immunotherapy, undoubtedly heralding boundless prospects for the therapeutic regimen of a wide range of solid tumors incorporating Hepatocellular Carcinoma in the times ahead.

Reference

1. H Sung, J Ferlay, RL Siegel, et al. CA Cancer J Clin. **71**(3):209-249.(2021)

2. A Baecker, X Liu, C La Vecchia, et al. Eur J Cancer Prev. **27**(3):205-212. (2018)

3. JX Yu,VM Hubbard-Lucey, J Tang. Nat Rev Drug Discov. **18**(11):821-822.(2019)

4. S Ma, X Li, X Wang, et al. Int J Biol Sci. **15**(12):2548-2560.(2019)

5. C Colovos, J Villena-Vargas, PS Adusumilli. Immunotherapy. **4**(9):899-902.(2012)

6. PLS Uson Junior, AJ Liu, MB Sonbol MB, et al. Chin Clin Oncol. **10**(1):11. (2021)

7. M Capurro, IR Wanless, M Sherman, et al. Gastroenterology. **125**(1):89-97. (2003)

8. Z Jiang, X Jiang, S Chen, et al. Front Immunol. **7**:690. (2017)

9. JR Gillespie, VN Uversky. Biochim Biophys Acta. **1480**(1-2):41-56.(2000)

10. H Liu, Y Xu, J Xiang, et al. Clin Cancer Res. **23**(2):478-488. (2017)

11. J Terris B, C Cavard, C Perret. J Hepatol. **52**(2):280-1.(2010)

12. K Kohga, T Tatsumi, T Takehara,et al. J Hepatol. **52**(6):872-9. (2010)

13. J Sun, ME Hemler. Cancer Res. **61**(5):2276-81. (2001)

14. CH Lamers, Y Klaver, JW Gratama, et al. Biochem Soc Trans. **44**(3):951-9.(2016)

15. DW Lee, R Gardner, DL Porter, et al. Blood. **124**(2):188-95. (2014)

16. CP Duong, JA Westwood, LJ Berry, et al. Immunotherapy. **3**(1):33-48.(2011)

17. K Minagawa, X Zhou, S Mineishi, et al. Pharmaceuticals (Basel). **8**(2):230-49. (2015)

18. T Gargett, MP Brown. Front Pharmacol. **5**:235. (2014)