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TOPIC REVIEW

Brain stem gliomas and current landscape

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Abstract

Purpose CNS malignancies are currently the most common cause of disease related deaths in children. Although brainstem gliomas are invariably fatal cancers in children, clinical studies against this disease are limited. This review is to lead to a succinct collection of knowledge of known biological mechanisms of this disease and discuss available therapeutics.

Methods A hallmark of brainstem gliomas are mutations in the histone H3.3 with the majority of cases expressing the mutation K27M on histone 3.3. Recent studies using whole genome sequencing have revealed other mutations associated with disease. Current standard clinical practice may merely involve radiation and/or chemotherapy with little hope for long term survival. Here we discuss the potential of new therapies.

Conclusion Despite the lack of treatment options using frequently practiced clinical techniques, immunotherapeutic strategies have recently been developed to target brainstem gliomas. To target brainstem gliomas, investigators are evaluating the use of broad non-targeted therapy with immune checkpoint inhibitors. Alternatively, others have begun to explore adoptive T cell strategies against these fatal malignancies.

Keywords Brainstem glioma · DIPG · Histone mutations · Therapeutic strategies

Introduction

Pediatric brainstem gliomas (pBSGs) are the most malignant pediatric brain tumors, and diffuse intrinsic pontine glioma (DIPG) is the most common brainstem glioma in pediatric patients, constituting 80% of all brainstem tumors [1]. DIPG cannot be surgically removed because of its infiltrative nature within a developing brainstem and, therefore, necessitates a therapeutic treatment option. While many combinatorial therapies have been tested in clinical trials, radiation therapy with concurrent and adjuvant temozolomide remains the current standard of care [2]. With limited treatment options, pBSGs constitute the leading cause of pediatric brain tumor-related fatalities [3] with a median survival of 10 months and a 2 year survival rate of less than 10% from clinical diagnosis [4, 5].

Recent neurosurgical advancements in stereotactic biopsies have provided patient-derived tumor samples for primary cell cultures and genomic studies. Modern genomic sequencing has revealed recurrent somatic mutations of the histone H3 genes (*HIST1H3B* and *H3F3A*) encoding p.Lys27Met that result in the global reduction of H3K27 trimethylation (H3K27me3) and inhibition of Polycomb Repressive Complex-2 (PCR2) methyltransferase [6–10]. Exclusively arising in pediatric high-grade gliomas (HGGs), these mutations predominate in midline structures, such as the brainstem, and are the hallmark mutations of pBSGs [7]. Other frequent somatic mutations in *ACVR1*, *PDGFRA*, and other histone H3 genes, *ATRX* and *TP53*, have been found in the genomic landscape of DIPG [11, 12, 6].

Although clinicians once believed pBSGs genetically resembled adult glioblastoma (GBM), these reoccurring mutations distinguish pBSG from its adult counterpart and may provide therapeutic targets for future research [13]. Current clinical trials are testing the efficacy of histone deacetylase inhibitors, BET inhibitors, Cdk4/6 inhibitors, and other small molecule inhibitors target these somatic mutations. Despite numerous emerging therapies, significant progress to prolong the median survival in pediatric patients has yet to be shown. Radiation therapy remains the only treatment modality for pBSGs that reduces symptomatic progression and prolongs progression-free survival [14–16].

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Clinical Diagnosis

The clinical presentation of pBSGs is determined by patient age, tumor growth rate, and tumor location within the brainstem [17, 18]. The median age of onset is approximately 6–7 years of age with younger pediatric patients having a lower median survival and are associated with more aggressive BSGs [19, 20]. Because of its rapid progression, patients with BSGs typically experience symptoms for less than a month prior to diagnosis [4]. At clinical diagnosis, patients may present with several symptoms including cranial neuropathy, deficits in vision, and ataxia [5]. Pediatric patients presenting with similar symptoms will undergo a MR imaging to diagnose the brainstem lesion. Depending on the tumor subtype, pBSG MR imaging findings will usually depict a pontine enlargement at the epicenter that can occupy more than 50% of the pontine cross-sectional area with DIPG potentially extending into the medulla [21–23]. Upon analysis, DIPG and other pBSG are hyperintense on T2-weighted and hypointense on T1-weighted images [24, 25].

Currently, stereotactic biopsies are not utilized in the U.S. standard care for DIPG patients. The genesis of non-invasive imaging replaced prognostic biopsies with MR imaging to diagnose and classify BSGs [26]. Physicians preferred diagnostic imaging over biopsies because of the minimized the risk to cause neurological damage and the additional histological data provided by biopsies would not influence future treatment of the patient due to limited treatment options. This aversion to prognostic biopsies created a dearth of brainstem tumor tissue, which inhibited comprehensive sequencing analysis of pediatric midline gliomas in the past. However, the development of the stereotactic headframe in the 1980s used MRI target coordinates to precisely extract a portion of the tumor sample with a side-aspirating biopsy needle, which resulted in a mortality rate of 0.7% [27–29]. While biopsies of the pons provided little information with heightened risk decades ago, stereotactic biopsies have provided valuable immunohistochemical diagnoses that can determine future treatment courses [30]. The decision of obtaining biopsy at the moment of diagnosis remains contentious. However, as investigation into the therapeutic targets of DIPG progresses, clinicians may begin implementing stereotactic biopsies into the U.S. standard care of DIPG.

pBSG H3K27M Mutations

Recent molecular and genetic data have found distinct genetic alterations that distinguish pBSGs from their adult counterpart and indicate that these tumors may arise from unique biological origins [13, 31]. Recurrent identification of histone H3 mutations in DIPG samples have revealed that both genetic and epigenetic alterations may drive DIPG tumorigenesis [6, 7, 31, 32]. Modern genomic sequencing has revealed high-frequency histone mutations in the H3 variants H3.1 and H3.3 that alter chromatin remodeling factors in pBSG. Somatic mutations in proteins that control the chromatin remodeling pathway often drive tumorigenesis [33]. These somatic mutations in histone H3 result in two mutually exclusive, distinct amino acid exchanges: a lysine 27 to methionine substitution (K27M) in variant H3.3 (*H3F3A* gene) and H3.1 (*HIST1H3B* and *HIST1H3C* genes) or a replacement of glycine by either arginine or valine (G34R/V) found exclusively in the variant H3.3 [6, 9, 32]. While G34R/V arise in pHGGs located in the cerebral cortex and are mainly associated with pGBM, the H3.3-K27M and H3.1-K27M mutations predominate exclusively in the midline that give rise to pBSGs [11]. Although the exact biological origin and repercussions of H3K27M are currently unknown, its high occurrence in DIPG elucidates its possibility of being an oncogenic driver mutation. Recent studies have found that 80% of DIPG tumors contain the characteristic K27M mutation in *H3F3A* and/or *HIST1H3B* [6, 7, 9, 32], which establishes p.LysMet as a potential molecular target for treatment (Fig. 1).

K27M mutations, in both the *H3F3A* gene that encodes histone H3.3 and the *HIST1H3B/C* genes that encode histone H3.1, substitute a lysine residue on the amino-terminal tail of H3.3, which biochemically inhibits polycomb repressor complex 2 (PRC2) [34]. Inhibition of PRC2 is dependent on the H3 tail contacting and altering the EZH2 subunit of PRC2, which can interfere with the role of PRC2 in gene silencing for stem cell differentiation and has been implicated in several cancers [35]. In humans, PRC2 comprises the substrate necessary for H3K27 trimethylation (H3K27me3) formation, while also inhibiting H3K27 acetylation (H3K27ac) [36]. Therefore, pBSGs with H3K27M mutations display a significant decrease in global H3K27me3 and a less dramatic increase in H3K27ac [9, 10, 37]. In fact, H3K27me3 reduction can be observed in 95% of DIPG, which is a more recurrent hallmark of pBSGs oncogenesis than the H3K27M mutation itself [34]. The dormancy of global trimethylation levels from other histone marks including H3K4, H3K9, and K3K36, indicates that the reduction of trimethylation levels is specific to the H3K27M mutation [9]. Acetylation and methylation of histone H3 at Lys 27 regulate its expression [36, 38]. Specifically, H3K27me3 is liked to chromatin structuring and gene repression, especially in genes associated in the development and differentiation of pluripotent stem cells [39]. This affinity for a neural precursor cell population in addition to the location-specific development of DIPG could indicate that H3K27M mutations may originate

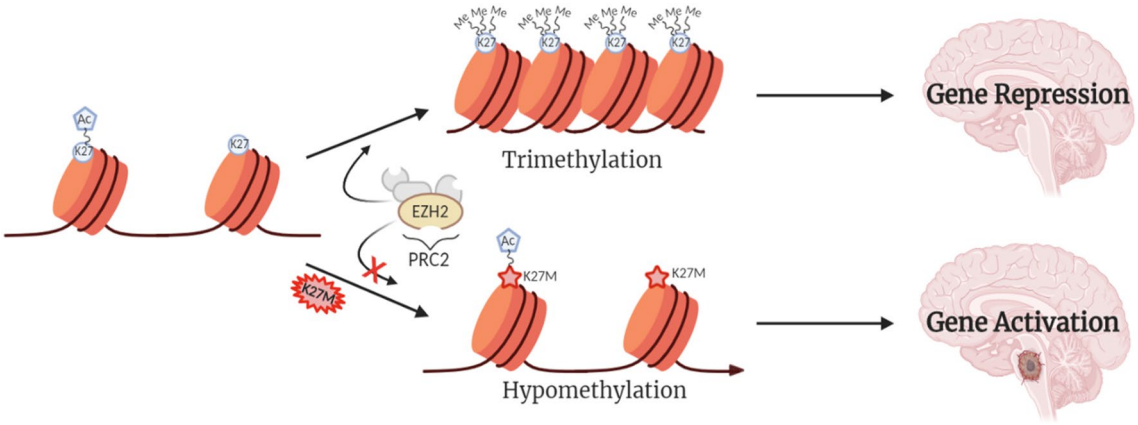

Fig. 1 Overview of H3K27 and its Epigenetic Modification. In normal neurological development, genes that regualte stem cell differentiation are silenced by the polycomb repressor complex 2 (PRC2). To repress these genes, the EZH2 subunit catalyzes the PCR2-mediated H3K27 trimethylation by binding to histone H3 tail, a common site for post-translational modification. However, in the H3K27M, the lysine substitute inhibits EZH2 binding which prevents PCR2-dependent methylation and results in aborrant gene activation

during neurological development. Novel findings have found a pontine precursor-like cell population that are Nestin$^+$ and Vientin$^+$ in the pediatric ventral pons region where DIPG develops, providing a possible DIPG cell of origin [40]. This cell population predominates during infancy and middle childhood, which overlaps with the median onset of DIPG as previously described [40].

pBSG Mutations (Non-H3K27M Mutations)

While current findings depict H3K27M mutation as the defining DIPG mutation, whole-genome sequencing has revealed several non-histone genetic alterations present in DIPG. Recent sequencing has revealed a recurrent, activating mutation in activin A receptor type I (*ACVR1*), which has been found in 20–30% of DIPGs [11, 32, 41]. *ACVR1* encodes for ALK2, a type I bone morphogenic protein (BMP) receptor known to phosphorylate SMAD1, SMAD5, and SMAD8 (SMAD1/5/8) in order to activate the BMP–TGF-β signaling pathway found in primary DIPG cultures [36]. Aberrant activation of BMP signaling can disrupt its regulation of cell proliferation in healthy tissues [42]. While somatic mutations in *ACVR1* have only recently been sequenced from DIPG tissue, they are the same germline mutations associated with fibrodysplasia ossificans progressive (FOP), an autosomal disorder of skeletal malformations which transforms soft connective tissue into bone tissue [43, 44]. Patients presenting with clinical features of FOP had identical heterozygous 617G➔A mutations (R206H) on codon 206, which is located at the end of the highly conserved glycine and serine activation domain [43]. Specifically, the R206H mutation induces a stronger gain of function when compared to its counterpart *ACVR1* mutants [45].

As expected, when DIPG cells were transfected with the p.R206H ACVR1 mutation, cells had greater activation of signaling through phosphorylated SMAD1/5/8 pathways without the presence of its ligand [36, 45]. Moreover, in vitro expression of p.R206H mutation has been shown to activate STAT3 signaling, a cell cycle and oncogenic promoter, and increases tumor incidence in combination with PDGFA signaling [46]. Occurring in less than 1% of other cancers, somatic mutations in *ACVR1* are highly specific to DIPG and coincide with wild-type *TP53* and the less prominent *HIST1H3B* mutation [31, 36]. DIPG cells expressing the Gly328Val ACVR1 had higher levels of phosphorylated SMAD1/5 when compared to those cells with wild-type *ACVR1* [32].

As the second most frequent mutation found in DIPG, loss-of-function *p53* mutations in the *TP53* gene were found in a majority of pBSGs mutant for H3.3-K27M [31]. These mutations arose with the same frequency in H3.3 wild-type DIPGs, indicating that the *p53* mutational frequency does not proportionally increase with H3K27M mutations [31]. Although the p53 mutation has been sequenced in over 50% of human cancers, its numerous targets involved in apoptosis, DNA repair, and cell cycle arrest have greatly complicated the developmfent of novel antitumor therapies for specific downstream targets [47]. *TP53* mutations confer resistance to p53-dependent apoptosis and allow tumor cells to acquire cross-resistance to the cytotoxic effects of conventional chemotherapy [48, 49]. In a study analyzing *TP53* mutational frequency in pHGGs, malignant gliomas in children >3 years of age more closely resembled gliomas of young adults associated with elevated *TP53* mutations when compared with tumors in children <3 years of age [10]. This finding coincides with other DIPG investigations that suggest distinct

molecular pathways of tumorigenesis depend on patient age [13, 31, 50].

The pBSG genomic landscape contains a *PDGFRA* locus amplification, which is present in 40% of the pBSGs with H3.3-K27M mutations [31]. *PDGFRA* is a receptor that stimulates cell signaling pathways involved in cell differentiation and is the predominant target of focal amplification in pBSGs. In relation to glioblastoma (GBM), *PDGFRA* is a defining feature in the Proneural glioma subtype that may arise from a neural stem cell [51, 52]. In the clinical setting, *PDGFRA* amplification transpires at a higher frequency in irradiation-induced tumors, suggesting that this genetic alteration contributes to childhood and adolescent tumorigenesis [53]. Moreover, *PDGFRA* locus amplification was found exclusively in tumors with H3.3-K27M mutations, indicating a possible downstream target of the defining mutation [34]. This evidence promotes the hypothesis that pBSGs may arise from a neural precursor cell origin.

Emerging Epigenetic Therapies

The recurrent genetic alterations found in a majority of DIPG tissue suggest an epigenetic mechanism for DIPG oncogenesis. Therefore, new therapies are being investigated to epigenetically modify the DIPG landscape to prevent or reverse tumor progression. Because global hypomethylation of H3K27 is the most common alteration found in DIPG, treatments that restore basal levels of H3K27 methylation may confer therapeutic benefit [54]. Reversing histone H3K27 demethylation can be achieved by inhibiting the K27 demethylases JMJD3 and UTX [55–57]. In a transformative study, GSKJ4, a cell permeable derivative of the H3K27 demethylase inhibitor GSKJ1, was shown to markedly increase K27 methylation which resulted in increased apoptosis of in vitro cells expressing H3K27M and displayed in vivo antitumor efficacy [54]. Interestingly, GSKJ4 displays sensitivity to K27M tumor cells with limited effectiveness against wild type glioma cells or cells expressing the H3.3 G34V variant [58]. These findings suggest that the antitumor efficacy of GSKJ4 is specific for K27M tumors.

Additionally, H3K27M was associated with elevated levels of acetylated H3K27 (H3K27ac) [10, 59, 60]. This epigenetic modification can be therapeutically targeted by inhibiting histone deacetylases (HDACs) [61]. HDACs remove acetyl groups from histone proteins which is associated with closed chromatin structure that prevents transcription. A chemical screening in multiple patient-derived DIPG cultures found that a particular HDAC inhibitor, panobinostat, reduced DIPG cell viability, prolonged survival, and synergized with GSKJ4 [62]. However, unlike GSKJ4, the activity of panobinostat was similar in both H3.3K27M and H3.3-WT cells. Panobinostat was verified to inhibit DIPG cell proliferation in vitro and, while it increased levels of H3 acetylation in the pons of tumor-bearing animals and restored H3K27 trimethylation, it did not correlate to an increased survival [63–65].

One of the difficulties translating HDAC inhibitors into clinic has been to confirm adequate penetration of the blood brain barrier (BBB) without causing adverse toxicity given the necessity of histone acetylation in healthy tissue. Currently, there are multiple clinic trials evaluating the efficacy of panobinostat and other HDAC inhibitors in DIPG patients (NCT03893487, NCT02717455, and NCT03566199). NCT03893487 is an early phase I clinical trial investigating the BBB perforation capability of fimepinostat, a small molecule that inhibits the activity of HDAC. NCT02717455 is also in its phase I to determine the dose-limiting toxicity and profile of panobinostat for patients with recurring DIPG. Both phase I clinical trials administer the HDAC inhibitor orally and, because of the BBB functional integrity, will have to balance drug permeability and toxicity. Convention-enhanced delivery (CED) represents a method of drug delivery by inserting catheter directly into the pons which reduces toxicity and avoids the BBB by infusing low amounts of water-soluble panobinostat directly into the brain parenchyma [66]. An ongoing clinical trial (NCT03566199) has developed a water-soluble formulation of panobinostat by encapsulating it in nanoparticles which they administer intratumorally by CED and are currently in a phase II study.

Immunotherapy

Directing the host immune system to induce potent antitumor responses has seen significant advances in prolonging patient survival in recent decades. However, this immunologic benefit has not currently translated into clinical trials for DIPG partly because of the immunosuppressive environment of the pons maintained to prevent inflammation [67, 68]. Despite these challenges of harnessing the host immune system to target DIPG, checkpoint inhibitors and adoptive T cell immunotherapy (ACT) have become promising approaches to mediate response in both early and advanced stages of the disease.

Immune checkpoint inhibitors (ICI) block co-inhibitory pathways that are activated by tumor cells to suppress T cell response against the malignancy which results in prolonged immune response in the tumor microenvironment. In particular, the programmed cell death (PD-1) pathway has been implicated in the pathogenesis of pediatric gliomas [69–71]. PD-1 is expressed on the surface of T cells to regulate their effector function which acts as a hardwired break for the adaptive immune system to prevent adverse cytotoxicity. However, cancer cells have hijacked this pathway by overexpressing the ligand of PD-1 (PD-L1) to evade

cytotoxic T cell function in the TME [72]. Monoclonal antibody therapies that block the PD-1/PD-L1 pathway can sustain the immune response against the malignancy [73] and extend median survival of HGGs when combined with radiation [74].

Despite its early promise, many cancer patients develop resistance to the treatment or do not derive therapeutic benefit [75–77]. Retrospective analyses conducted on anti-PD1 therapy used to treat DIPG have shown to neither confer efficacy by itself [78] nor enhance the survival benefit of re-irradiation [79]. Still, clinical trials are being conducted to treat young children with recurrent gliomas by using anti-PD-1 monoclonal antibodies. NCT02359565 is a phase I clinical trial testing the efficacy of pembrolizumab, an anti-PD-1 antibody, for patients with DIPG or other HGGs, while NCT03690869 is implementing the PD-1 inhibitor REGN2810 in conjunction with radiation to treat relapsed CNS tumors.

In addition to checkpoint inhibition, ACT has seen promising advances in the treatment of malignant gliomas [80, 81]. ACT is the isolation and expansion of tumor-reactive T cells ex vivo which are infused back into the patient (Fig. 2).

As a promising form of ACT, chimeric antigen receptor (CAR) T cells have had great success in treating refractory B cell malignancies, which has not translated well into solid tumor models [82, 83]. CAR T cells work by antigen-specific direct killing of cells. It has been shown that multiple antigen-targeting is more effective than single antigen. CAR T cells produced through gene editing software such as TALEN and CASPER. New antigens associated with high-grade gliomas may provide suitable targets for CAR T cells including the membrane protein B7-H3 (PD-L1) [84–86] or the disialoganglioside GD2 that is highly expressed in midline gliomas [87]. In a preclinical study, GD2-directed CAR T cell therapy mediated a potent antitumor response in mice bearing patient-derived diffuse midline glioma orthotopic xenograft models and was well-tolerated [87]. This finding has been translated in an ongoing clinical trial (NCT04099797) to determine the adequate dosing of a C7R-GD2 CAR T cell therapy, an anti-GD2 CAR T cell modified with the *CR7* gene to provide a constant supply of cytokines to the T cell.

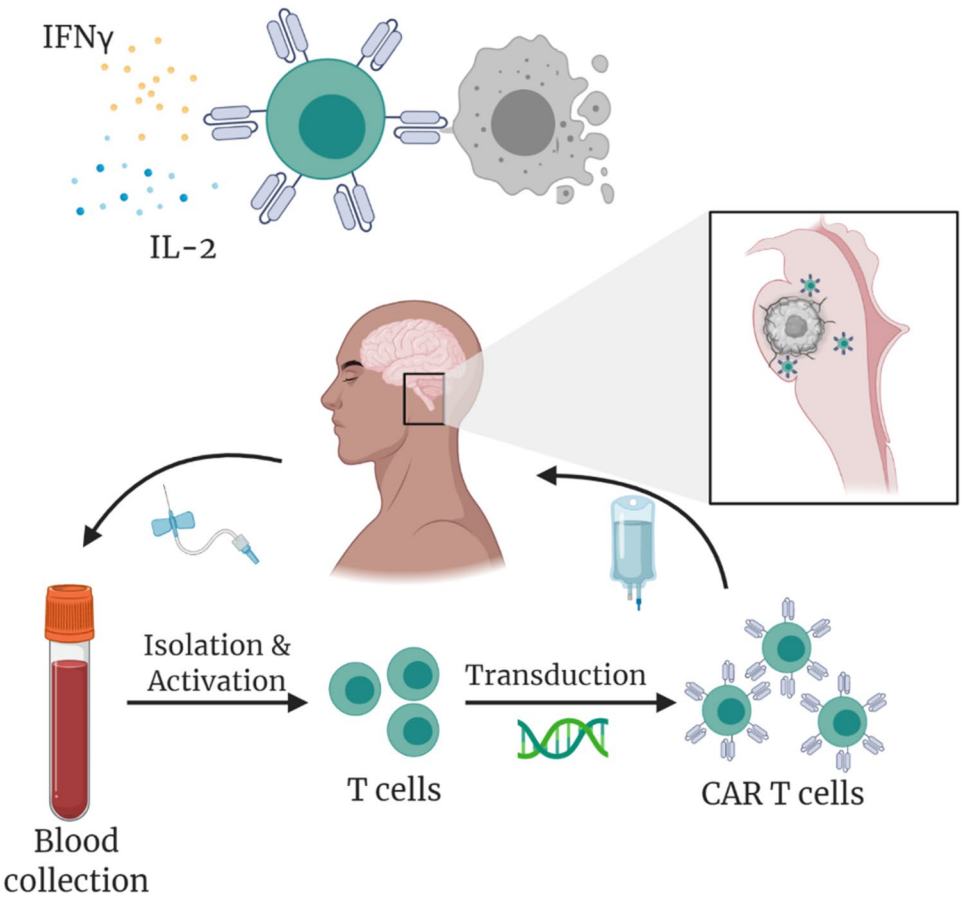

Fig. 2 Generation of CAR T cells targeting BSGs. The develoopment of CAR T cell therapy begins with the collection of a patient's peripheral blood mononuclear cells. The cells are enriched for a T cell susbset, such as a $CD3^+$ population, and subseqently expanded and activated ex vivo using costimulatory ligands such as CD28. Activated T cells are then genetically modified by electroporation or viral vectors, such as lentiviral or retroviral vectors, to deliver the CAR gene. Then, the CAR T cells are actived against the specific tumor target and expanded ex vivo using costimulatory ligands before administration to the patient

Summary

pBSGs are a subset of highly aggressive cancers that currently have limited treatment options with DIPG representing the leading cause of pediatric brain cancer fatalities. Although no therapies for DIPG or other pBSGs have been shown to consistently extend survival beyond that of radiation therapy, advancements in understanding its epigenetic dysregulation, despite the scarce availability of tumor tissue, may signify the possibility for future therapeutic breakthroughs. Recent research characterizes DIPG as biologically distinct from adult high-grade gliomas with hallmark mutations that contribute to its molecular pathogenesis. Promising therapies such as HDAC inhibitors that epigenetically modify the tumor landscape to prevent progression are currently in clinical trials. In addition, ongoing research into harnessing the host immune system through PD-1 monoclonal therapy and CAR T cells have shown preclinical success and have been translated into clinical studies.

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Compliance with ethical stadards

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