



Multimodal neuro-nanotechnology: Challenging the existing paradigm in glioblastoma therapy

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Integrating multimodal neuro- and nanotechnology-enabled precision immunotherapies with extant systemic immunotherapies may finally provide a significant breakthrough for combatting glioblastoma (GBM). The potency of this approach lies in its ability to train the immune system to efficiently identify and eradicate cancer cells, thereby creating anti-tumor immune memory while minimizing multi-mechanistic immune suppression. A critical aspect of these therapies is the controlled, spatiotemporal delivery of structurally defined nanotherapeutics into the GBM tumor microenvironment (TME). Architectures such as spherical nucleic acids or poly(beta-amino ester)/dendrimer-based nanoparticles have shown promising results in preclinical models due to their multivalency and abilities to activate antigen-presenting cells and prime antigen-specific T cells. These nanostructures also permit systematic variation to optimize their distribution, TME accumulation, cellular uptake, and overall immunostimulatory effects. Delving deeper into the relationships between nanotherapeutic structures and their performance will accelerate nano-drug development and pave the way for the rapid clinical translation of advanced nanomedicines. In addition, the efficacy of nanotechnology-based immunotherapies may be enhanced when integrated with emerging precision surgical techniques, such as laser interstitial thermal therapy, and when combined with systemic immunotherapies, particularly inhibitors of immune-mediated checkpoints and immunosuppressive adenosine signaling. In this perspective, we highlight the potential of emerging treatment modalities, combining advances in biomedical engineering and neurotechnology development with existing immunotherapies to overcome treatment resistance and transform the management of GBM. We conclude with a call to action for researchers to leverage these technologies and accelerate their translation into the clinic.

glioblastoma | neuro-nanotechnology | immunotherapy

GBM (glioblastoma) is a highly aggressive form of brain cancer that is infiltrative in nature. The poor survival rates of GBM patients and the challenges in identifying effective treatments are due, in part, to the low abundance of effector immune responses in the TME (tumor microenvironment), the predominance of immunosuppressive myeloid cells, and the presence of the blood-brain barrier (BBB) that limits the intratumoral accumulation and efficacy of most systemically administered immunotherapies. The identification of new multi-modal immunotherapies capable of increasing T cell trafficking and activation, re-educating myeloid cells,

penetrating the BBB, and overcoming multi-mechanistic treatment resistance are critical to transforming the clinical management of GBM. Here, we summarize the major challenges in immuno-oncological drug development and implementation in the context of GBM and highlight the most recent advances in developing multimodal nano-immunotherapeutics and their promise in GBM therapy, particularly when combined with companion surgical techniques and existing immunotherapies to boost their efficacy.

1. Challenges in GBM Treatment: Current Therapeutic Strategies and Roadblocks

The standard of care for GBM consists of maximal surgical resection, chemotherapy, radiation, and the application of tumor-treating fields. Due to the invasive nature, the substantial inter- and intratumoral heterogeneity, the regenerative

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capacity of treatment-resistant cancer stem cells, and challenges in achieving high concentrations of therapeutic agents in the central nervous system (CNS), these therapies have only modestly improved patient survival. Immunotherapies are a promising modality for the treatment of GBM because of their potential to stimulate tumor-specific immune effector cells that can penetrate deep into the tissue to eliminate infiltrative GBM cells while limiting damage to surrounding healthy cells. However, the majority of GBM patients do not derive sustained clinical benefits from current strategies due to the inability of immunomodulatory agents to accumulate at therapeutic levels in tissues where anti-tumor immune responses are generated—including the TME—and to overcome the numerous immunosuppressive mechanisms that limit immunotherapeutic efficacy.

1.1. Physiological Barriers. GBM is shielded by the BBB, which consists of an extracellular matrix, specialized endothelial cells, pericytes, and astrocytes that work in concert to stringently regulate the entry of cells, macromolecules, and ions into the CNS (1). As such, most systemically administered small molecules, antibodies, and cell therapies are excluded from accessing the brain (2). GBM is characterized by substantial neo-angiogenesis driven by tumor cell-secreted pro-angiogenic factors, like vascular endothelial growth factor (3). These newly formed vessels within the tumor exhibit complex networks and heightened permeability. Additionally, glioma cells can infiltrate and co-opt existing brain vasculature and transdifferentiate into endothelial cells or pericytes (4–6) to form the blood-tumor barrier (BTB). The BTB, compared to the BBB, is more permeable due to transcriptomic and structural alterations, such as reduction in tight junction proteins and reconfiguration of astrocytic end-feet (7–9). These regional differences in vascular heterogeneity can account for disparate drug concentrations, distribution, and target engagement (9).

Many modern strategies for drug delivery into the brain focus on disrupting tight junctions between endothelial cells. These disruptions, achieved through mechanical and pharmaceutical methods, aim to increase the diffusion of substances throughout the TME. Osmotic disruption by administering hypertonic solutions improves BBB penetration but is limited by procedural complexity and some adverse neurological outcomes likely due to nonspecific and extended duration of barrier disruption (10, 11). Tight junctions in the BBB can also be modulated through the action of pharmacologic agents. Still, previous trials showed no significant increase in efficacy, highlighting the need for alternative approaches (12). Focused ultrasound (FUS) has emerged as a promising strategy that utilizes transcranial delivery of low-frequency ultrasound waves, resulting in transient and targeted BBB permeability. Preclinical studies have shown that FUS can improve the delivery of nanoparticles (NPs), antibodies, and DNA to the brain and enhance the efficacy of immunotherapies (13). FUS is safe in human subjects (14, 15) and allows for repeated administration of cytotoxic drugs into the brain (14).

Productively engaging the transcellular pathway is not trivial and typically involves the modification of drugs, such as incorporation into NPs or conjugation to receptor-mediated transcytosis (RMT) ligands, antibodies, or targeting peptides

(16–18). Of the cerebral vasculature receptors that have been studied to enhance RMT, the transferrin receptor (TfR) is the most thoroughly evaluated. Despite the encouraging progress, the extent to which engaging TfR enhances transcytosis is debated, as most endocytosed material is recycled back to the luminal side of endothelial cells (19). Further characteristics that enable non-invasive BBB permeability of nanostructures for GBM treatment include the enhanced permeability and retention effect, active receptor targeting, adsorption mediated transport, and passive diffusion of small lipophilic particles (20). For example, fluorescently tagged protein NPs have shown efficacy in their ability to traffic to the CNS and infiltrate the TME while safely targeting macrophages in the CNS (21). Additional characteristics that impact NP BBB permeability include multivalency, which enables the conjugation of multiple payloads and targeting moieties for receptor-mediated endocytosis (22, 23); NP responsiveness to physical forces, which can enhance their BBB permeability in response to external stimuli like FUS (24) and photothermal laser stimulation (25); the tunability of NP shape, surface chemistry, and size, which allow for attachment (26–28) or internalization (29, 30) of particle-based therapies in endogenous or engineered immune cells, improved pharmacokinetics via coatings (31), or size-based deposition (32).

Alternative approaches consider bypassing the BBB using local delivery methods, including drug administration into the tumor resection cavity (33). Biodegradable polymeric wafers carrying the alkylating agent carmustine (Gliadel™) deliver chemotherapy directly into a tumor resection cavity. Gliadel™, however, showed only marginal anti-tumor efficacy, partly due to a lack of interaction between the drug and the wafer, the limited tissue penetration of encapsulated cargo, and the lack of immune modulation (34–38). Local and sustained delivery approaches that modulate the immune microenvironment and kill cancer cells have the potential to achieve long-lasting antitumor effects.

1.2. Infiltrative and Heterogeneous Disease. GBM is an inherently heterogeneous and infiltrative disease that cannot be cured with surgical intervention alone. In many cases, the extent of surgical resection is limited by tumor location in eloquent brain areas that are involved directly in motor-sensory control, language, vision, and memory functions (39). The remaining, diffusely infiltrating component causes inevitable tumor recurrence (40). Studies have characterized the extent of neoplastic infiltration, observing that gliomas spread through white matter tracts to the brainstem in 82% of patients (41).

In addition to the pervasive infiltration of normal brain parenchyma (41), GBM displays an astonishing degree of inter- and intra-tumoral heterogeneity. Next-generation sequencing demonstrated that GBM harbor aberrations in one of three signaling axes, including the p53, retinoblastoma, or mitogen-activated protein kinase (MAPK) pathways, exist in three transcriptionally defined subtypes (proneural, classical, and mesenchymal), and exhibit plasticity in transitioning between them (42, 43). The tumor transcriptional subtype determines tumor cell-intrinsic biological properties and modulates TME immune cell infiltration and activation. The mesenchymal subtype, for example, is enriched for alternatively activated, immunosuppressive (“M2”) macrophages and depleted for activated natural killer (NK) cells (42). In

addition, single-cell sequencing and spatial transcriptomics/proteomics studies revealed a high degree of intrinsic intratumoral heterogeneity, characterized by the presence of clonal and sub-clonal differentiated tumor cell populations, glioma stem cells (GSCs), which can generate differentiated daughter cells and play central roles in disease pathogenesis, recurrence, and therapeutic resistance (44–47).

The high degree of tumoral heterogeneity, together with the presence of therapy-resistant GSCs, and the resistance to radiation treatment, chemo- and targeted therapeutics, indicate that immunotherapeutic strategies, wherein effector cells can access the entire parenchyma, are needed for improved tumor control and elimination of distant microscopic disease.

1.3. The Immunosuppressive GBM TME. The implementation of immunotherapeutic strategy to blunt GBM progression is challenging because there are few T cells within the GBM TME due to bone marrow sequestration; in addition, those that are present are exhausted and refractory to immune checkpoint inhibitor modulation (48, 49). Other immune effector cells, such as NK cells, are also deprogrammed from exerting anti-tumor activities (50). The second significant obstacle to immunological reactivity is multi-mechanistic iatrogenic and tumor-mediated immunosuppression. Systemic immune dysregulation is evident in GBM patients regardless of their treatment status (51, 52). Like other types of cancers, GBM alters the expression of immunomodulatory surface ligands to promote a pro-tumor environment, such as the downregulation of major histocompatibility complex class I (MHC I) and upregulation of PD-L1, and in doing so, inhibits effector responses of cytotoxic T cells (53, 54). In addition to the low frequency and functional exhaustion of intratumoral T cells, the high abundance of immunosuppressive macrophages and/or microglia represents another suppressive mechanism. Patients with GBM present with hematologic abnormalities, including severe reductions in overall CD4+ T cell populations and reduced expression of MHC II on circulating monocytes (51, 55, 56). In both tumor-bearing patients and mice, lymphoid organs are markedly reduced in size compared to matched naive controls (48, 57).

It has become evident that therapeutic strategies must productively reprogram the GBM TME and the host's immune system to substantially improve outcomes. The unique approaches currently being clinically evaluated include the use of immune checkpoint inhibitors (ICI), therapeutic vaccines, dendritic cell vaccines, oncolytic viruses, chimeric antigen receptor (CAR) T cells, and combinations thereof (58–64). CAR T cells are rapidly evolving in immunotherapy of GBM (65), with many clinical trials showing efficacy following recognition of tumor-associated antigens, such as EGFRvIII (66), HER-2 (67), or IL13Ra2 (68). In preclinical models, next-generation CAR therapies have employed synthetic genetic logic circuits to address issues with broad antigen expression and tumor heterogeneity (69). However, exhaustion of CAR T cells can reduce their capacity for persistent anti-tumor action (70). ICIs have become the standard of care in a selection of solid tumors, including advanced melanoma and non-small cell lung cancer. In contrast to these immunogenically "hot" tumors with high mutational burdens, GBM is considered "cold," characterized by the emergence of intrinsic and

adaptive resistance mechanisms to therapy (71). As such, clinical trials of first-generation ICIs in GBM demonstrated that only a small fraction of patients benefited from treatment (72). Anti-PD-1 and anti-CTLA-4 therapeutics require activated T cells, which are mostly deficient within the GBM TME but may also require local rather than systemic ICI administration to maximize therapeutic benefit. Because the GBM TME is myeloid-dominant, effective strategies that employ ICIs will likely require combinatorial approaches that also target innate immune cell targets.

1.4. High-Priority Immune Targets in GBM. Myeloid cells must be reprogrammed to potentiate effector T cell tumoricidal activity because these cells produce immune suppressive cytokine and chemokines in the GBM TME to increase tracking and activation of effector T cells (73, 74). In recent years, NPs have been shown to induce the repolarization of anti-inflammatory M2-type macrophages toward a proinflammatory M1 type, which can amplify immune responses and sensitize tumors to (T cell-targeting) immunotherapies (75, 76). Indeed, researchers have begun exploring molecular targets in myeloid cells, particularly the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway, signal transducer and activator of transcription 3 (STAT3) signaling, and components of the adenosine pathway, such as CD73 (63, 77, 78). The cGAS-STING pathway is one of the primary immune sensing mechanisms that bridges the innate and adaptive immune systems. Upon its recognition of tumor-derived double-stranded (ds) DNA, cGAS produces the cyclic dinucleotide (CDN) cGAMP, which binds to STING, inducing the expression of type I interferons (IFN) and pro-inflammatory cytokines by activating interferon regulatory factor 3 and nuclear factor- κ B signaling (79, 80). The expression of type I IFNs and pro-inflammatory cytokines promote T cell effector function and trafficking to the tumor and repolarize immunosuppressive myeloid-derived suppressor cells (MDSCs) and macrophages (81). Intratumoral administration of CDNs inhibits tumor growth in cancer models, including glioblastoma (GBM) (82) and is currently being tested in clinical trials for patients with advanced extracranial cancers. Like other immunotherapy targets, cGAS-STING-driven immunity is antagonized by immunosuppressive mechanisms, including those driven by the activation of STAT3, a master transcriptional regulator of oncogenic signaling and anti-tumor immunity. STAT3 enhances the accumulation of immunosuppressive myeloid cells, thereby inhibiting effector T cell function (83). Together with recent studies identifying STAT3 inhibitors as a promising class of immunotherapeutics (63, 84, 85) that enhance anti-tumor immunity in response to STING pathway activation (86), these findings suggest that the combination of cGAS-STING pathway agonists with STAT3 inhibition may synergistically enhance immune responses within the GBM TME (87, 88).

Profiling the expression of immunomodulatory targets on peripheral and tumor-infiltrating immune cells has demonstrated that PD-1 and adenosine pathway components, including CD73, are the most frequently expressed immunosuppressive factors in glioma patients compared to healthy individuals (89). GBM tumor cells escape immunosurveillance by triggering the PD-1 immune checkpoint pathway in T cells. During treatment with anti-PD-1 monoclonal antibodies, antigen-specific effector T cells expand in peripheral immune

compartments and secondary lymphoid organs and then infiltrate the GBM TME (90). Since PD-1 is also expressed in tumor-associated myeloid cells, including MDSCs, tumor-associated macrophages, and dendritic cells, myeloid-specific PD-1 inhibition further augments anti-tumor immunity despite sustained PD-1 expression in T cells (91). These data suggest that PD1 blockade acts on peripheral and tumor-associated immune systems. Like PD-1, the co-targeting of CD73 on tumor cells and host immune cells is required for optimal outcomes of CD73-targeted therapy, as shown for extracranial cancers (92). Recent studies indicate that an immunosuppressive CD73^{hi} myeloid subset persists in GBM patients who receive anti-PD-1 therapy and that CD73-deficient mice show therapeutic benefit from systemic treatment with anti-PD-1 (78, 93). These data credential CD73 and PD-1 as high-priority combinatorial immune targets in GBM.

2. Emerging Advances in GBM Immunotherapeutic Treatment

Delivering therapies to modulate high-priority immune targets requires state-of-the-art materials with biophysical properties enabling privileged drug access to the cells and tissues of interest. In this regard, nanostructures are ideal as they allow one to modularly present multiple components to maximize efficacy. For this article, we focus on two prime examples. In addition to their favorable delivery profiles, modular nanotechnologies like spherical nucleic acids (SNAs) and poly-beta amino esters (94) can be designed with secondary functionality to engage the desired target. Research into the optimal administration and dosing of immunotherapies has shown that spatiotemporally controlling drug release is a key to achieving optimal therapeutic effect. Advanced materials for local drug release will enhance therapeutic efficacy and tolerability by concentrating therapy directly in the target tissue and enable sustained and local delivery of nanotherapeutics for maximum tissue penetration and efficacy. Such an approach will trigger the effective activation, trafficking, and maintenance of pro-inflammatory immune cells in the GBM TME, circumvent the need for repeat dosing, and permit the delivery of combination therapies that require different spatiotemporal release profiles to concomitantly target multiple mechanisms of immune suppression (71). Recently, materials like dextran-based hydrogels (95), can be administered to the tumor resection cavity and used to release drugs with optimized kinetic profiles. Finally, combining the controlled local release of nanotherapeutics with surgical techniques for minimally invasive tumor ablation, such as laser interstitial thermal therapy (LITT), in which a laser probe causes hyperthermic tumor cell death and increases local BBB permeability, may help to maximize immunotherapeutic effectiveness. LITT exerts several effects, including tumor tissue debulking that will likely enhance the potency of immunotherapies in the short term through the release of damage-associated molecular patterns (DAMPs), tumor-derived exosomes, and potentially antigens and, in the long term, by increasing the access of systemically primed immune cells to the TME (96). In subsequent sections, we describe a strategy for developing modular neuro-nanotechnology to overcome the (immuno-) therapeutic resistance of GBM.

2.1. Local Hydrogel-Mediated Delivery of Nano-STING Agonists to Enhance Long-Term Anti-Tumor Immunity. Hydrogel technologies have emerged as promising adjuvants to surgical resection or tumor biopsy for delivering molecular, cellular, and nanoscale drugs to brain tumors. Hydrogels bypass the BBB and control the spatiotemporal release of multiple therapies following a single injection (97–99). This approach further allows for the concentration of therapies locally, helping to revert immunosuppressive mechanisms within the TME, stimulate anti-tumor immunity, and prevent the immunosuppressive effects of existing therapies (100, 101). This also improves tolerability by reducing drug distribution to off-target sites, making therapy administration possible during the critical window between surgical resection and the initiation of chemoradiotherapy. Adhesive hydrogels are conducive to this approach as they can be designed to coat complex tissue surfaces generated during surgery through spraying or injection, facilitating precise targeting of residual tumor cells (102, 103). The structure and chemistry of hydrogels can also be tailored to control the release profile of associated therapies, enabling optimal dosing kinetics that may not be feasible with other administration modes (104). Moreover, the three-dimensional porous structure of hydrogels can promote the recruitment and infiltration of cells (105), including immune cells, for controlled interactions or manipulation, facilitating the uptake of embedded therapies and enhancing their effectiveness (106). Hydrogels can be engineered to mimic the mechanical properties of native tissue, minimizing the potential for foreign body responses and implant fibrosis (107). Finally, hydrogels can encapsulate therapies, protecting them from degradation and providing a depot for cell-based therapies or the local engineering of these entities (108, 109).

As an example, the Artzi lab has developed an innovative technology using adhesive dextran-dendrimer hydrogels for the controlled delivery of therapies to solid tumors (Fig. 1 A, *i*). These hydrogels can be applied by spraying or injection and undergo *in situ* gelation, providing advantages over other hydrogel formulations. The adhesive nature of these hydrogels minimizes material displacement or fragmentation, reducing the risk of adverse events and improving efficacy. Extensive work has demonstrated that material formulation can be tuned to achieve adhesion in different tissue microenvironments, including inflammatory and neoplastic lesions where the tissue surface chemistry and immune infiltrate have been modified by disease state-specific pathologic processes (Fig. 1 A, *ii*) (95). These hydrogels have been used over the past decade to deliver a wide range of therapeutic agents to solid tumors, including small molecules, antibodies, nucleic acids, and nanomedicines (110–112). Local administration of hydrogels has shown superior efficacy compared to traditional systemic delivery methods (113). The multivalency of these dendrimer and dextran polymers facilitates the integration of therapies onto the hydrogel network, enabling their continuous release as the hydrogel degrades. Given these properties, the use of adhesive hydrogels for intracranial delivery of therapies targeting GBM, such as CDN-NPs (114) recently developed by the Artzi group to activate the cGAS-STING pathway, holds promise in blunting immunosuppression in the GBM TME. These particles reduced myeloid-mediated immunosuppression in the TME of different

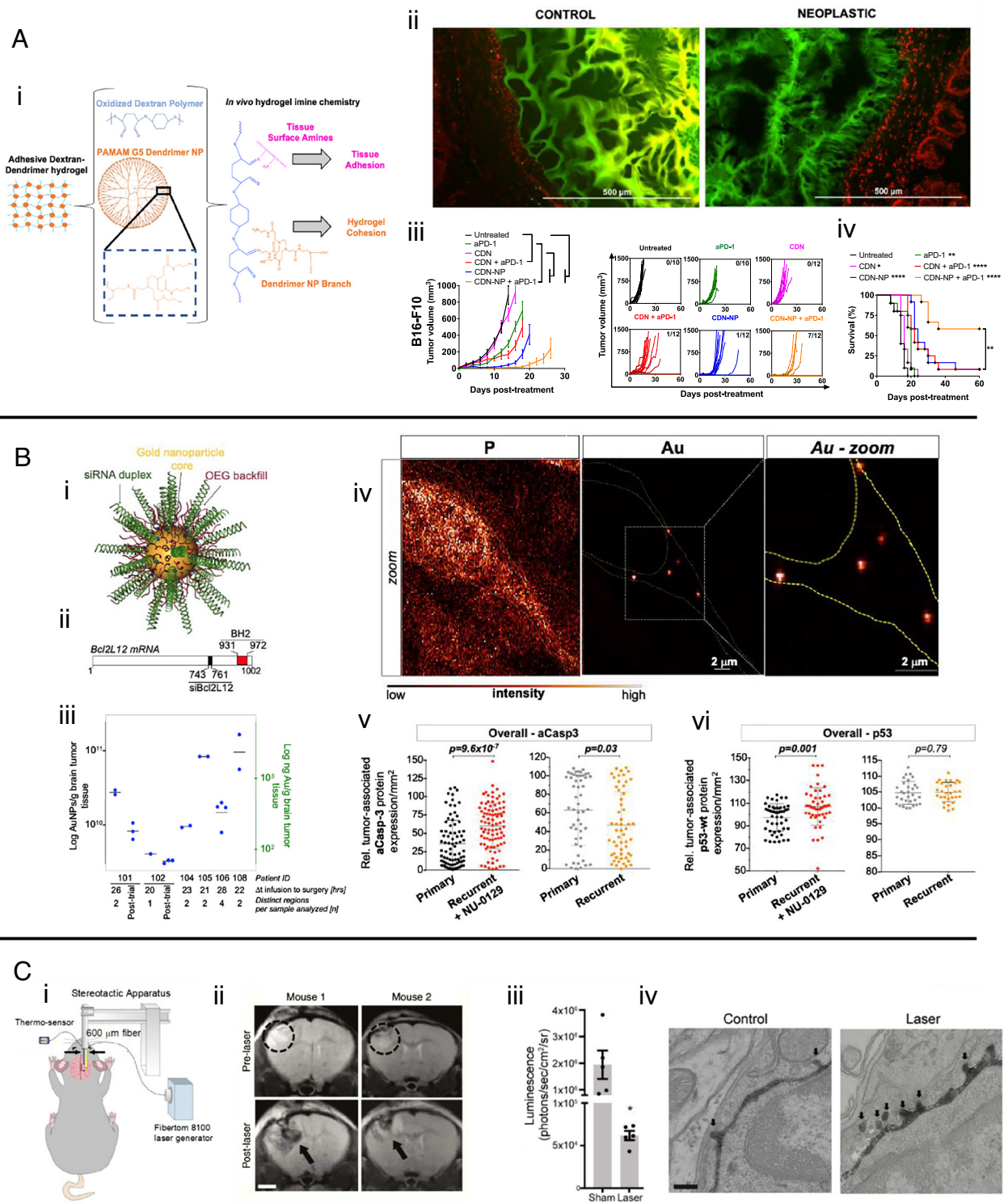


Fig. 1. (A) Potential of locally administered adhesive hydrogels combined with immunostimulatory NPs for anti-cancer therapy. (i) Schematic showing the chemical structure of adhesive hydrogels formed from dendrimer nanoparticles and oxidized dextran and their beneficial in vivo properties. (ii) Confocal images showing tissue (red) interactions of implanted hydrogels (green) in healthy and neoplastic tissue. (iii) Tumor volume and percent survival of B16-tumor bearing mice treated with CDN-NPs or CDN alone in combination with anti-PD-1 antibodies (iii, iv) reproduced from ref. 94. (B) Gene-regulatory SNAs as an Emerging Therapeutic Modality for GBM. (i) Schematic of the modular SNA architecture (NU-0129). (ii) *Bcl2L12* mRNA with binding site for the siRNA oligonucleotide used to functionalize gold nanoparticle cores (nucleotide position 743 to 761; black box). Positions for nucleotides encoding the C-terminal BH2 are indicated (nucleotide position 931 to 972; red box). (iii) ICP-MS analysis of bulk patient GBM tissue, including tumor recurrences post NU-0129 trial enrollment for patients 101 and 102; tumors recurred 159 and 174 days post NU-109 trial enrollment, respectively. N, number of tumor regions sampled. Δt , time from surgery to infusion. Shown is the median. (iv) XFM-Bionanoprobe assessment of patient tumor reveals extranuclear/cytoplasmic distribution of Au in tumor cells. (v, vi) Protein expression of active caspase-3 and wild-type p53 in matched newly diagnosed and NU-0129-treated recurrent GBM. Quantification of overall IHC staining intensities for the *Bcl2L12* downstream effectors active caspase-3 and wild-type p53. From ref. 129. Reprinted with permission from AAAS. (C) LITT Productively Modulates the BBB in Models of GBM. (i) Schematic of murine LITT administration. (ii) T2-weighted MR images of LITT-treated tumor-bearing mice. (iii) Quantification of tumor volume using bioluminescence imaging posttreatment. (iv) Representative transmission electron microscopy images following intravenous HRP administration in the sham and laser-treated brain (post-laser day 3). Arrows indicate HRP-filled vesicles within endothelial cells. (Scale bar, 100 nm.) Reproduced from ref. 155, Oxford University Press.

tumor types, including melanoma, colon and breast cancers, and increased the recruitment and activation of anti-tumor immune effector cells, such as cytotoxic T and NK cells (94). Furthermore, they have been optimized for enhanced biocompatibility and robust cytoplasmic delivery of CDNs, resulting in potent immune cell activation and extended survival in multiple tumor models (Fig. 1 A, *iii* and *iv*) (94, 114). While local polymer-based delivery systems (e.g., Gliadel™) (36) and other preclinical delivery systems have previously been explored, numerous challenges still exist, including poor control over spatiotemporal drug release and adhesive mismatch between the implant and brain tissue (37). Advances in hydrogel-based technologies that consider the design of tissue-material and drug-material interactions could address many of these challenges and expand the therapeutic arsenal available to patients with GBM. Therefore, we consider the preclinical and clinical development of dextran-dendrimer hydrogels that can achieve rapid gelation and tissue adhesion following administration into the tumor bed or resection cavity to deliver combinatorial regimens as a key area of neuro-oncology research.

2.2. Multimodal Immunostimulatory SNAs for cGAS Activation and STAT3 Suppression. SNAs represent a powerful class of nanotherapeutics that consist of a NP core functionalized with a shell of densely packed, radially oriented oligonucleotides (ODNs) (Fig. 1 B, *i*). ODN-based therapeutics for immune stimulation have emerged as a potent cancer treatment approach (117, 118). However, the delivery of nucleic acid payloads to tumor sites remains challenging because unmodified ODNs are rapidly degraded by serum RNases and DNases and have a poor cellular uptake (119). SNAs have properties vastly different from linear nucleic acids of the same sequence (120, 121). Unlike linear nucleic acids, SNAs enter many cell types in high quantities without auxiliary transfection agents. This rapid cellular uptake is mediated by pattern recognition receptors on the cell surface (namely class A scavenger receptors) through caveolae-mediated endocytosis, while the pronounced nuclease resistance is achieved through steric blockade mediated by the dense oligonucleotide corona (122). Countless iterations have shown that SNAs are highly tailorable nanoarchitectures (119, 123, 124). The ability to fine-tune oligonucleotide and core identities has been critical for developing SNAs as anti-cancer therapeutics (119, 125) and diagnostic tools (126–128), with many architectures commercialized and undergoing clinical testing. Early-phase clinical trials of gene-regulatory gold-SNAs carrying small interfering (si) RNA oligonucleotides in patients with recurrent GBM treatment (NCT03020017) were led by the Stegh laboratory (Fig. 1 B, *ii*, *iii*, *iv*, and *v*). When delivered intravenously, SNAs designed to silence the expression of Bcl2Like12 (drug moniker: NU-0129), a p53-destabilizing oncoprotein (Fig. 1 B, *ii*), accumulated in tumors (Fig. 1 B, *iii*) and were detected in tumor-associated macrophage, endothelial cells, and within the cytoplasm of intraparenchymal tumor cells (Fig. 1 B, *iv*). Further, SNAs reduced tumor-associated Bcl2L12 and enhanced active caspase-3 and p53 protein levels (Fig. 1 B, *v*) (129). This treatment was shown to be safe for use in non-human primates and GBM patients and was not associated with adverse side effects.

Immunostimulatory SNA constructs, with CpG oligonucleotides (CpG-SNA), exhibit remarkable efficacy against murine

lymphoma due to their multivalent, high affinity binding to Toll-like receptor 9 for triggering innate immune response (115). The core size, surface curvature, oligonucleotide, and anchor chemistry can be tuned to enhance TLR-agonistic activity (130). CpG-SNAs are safe in healthy subjects, as evaluated in a phase I study using single and multiple-dose regimens (NCT03086278). A phase Ib/II clinical trial evaluated the safety, tolerability, and efficacy of SNAs administered intratumorally, alone or in combination with ICIs, in patients with Merkel cell carcinoma or other advanced solid tumors (NCT03684785) (131–133). Tumor shrinkage was observed in 37% of patients, with a 33% overall response rate at the highest dosage. Additionally, SNAs can be formulated with various DNA adjuvants to engage different immune pathways more potently than linear ODNs of the same sequence (134–136).

Other NP types also offer a versatile platform for activating the STING pathway and enhancing immunotherapeutic efficacy (94, 137, 138). In addition to using CDN-loaded nanostructures as described in the previous section, we envision SNAs as a first-in-class immunotherapeutic to deliver oligonucleotides that target the cGAS enzyme upstream of STING. The activity of cGAS is catalytic (i.e., activating one cGAS enzyme will produce many-fold more cGAMP molecules), and its activation is therefore expected to more robustly activate the cGAS-STING pathway compared to treatment with exogenous synthetic CDNs. Emerging data also suggest pro-inflammatory functions of cGAS that are independent of STING including activation of NLRP3 inflammasomes (139), which results in the production of IL-1 β and IL-18 cytokines that recruit and activate NK and effector T cells (140). This suggests that cGAS agonists are likely to stimulate a broader range of pro-inflammatory signaling in comparison to therapeutics that are limited to STING activation.

Certain properties of dsDNA that drive the potency and activation of cGAS have been described previously and include ODN length and orientation (141–143). Specific structural characteristics of ODNs and their arrangement on NP cores are unknown and need to be further explored. Because the cGAS enzyme binds to DNA agnostic of its sequence, SNAs can be formulated with bifunctional dsDNA (bi-SNAs) that not only activate cGAS but in addition can act as decoy sequences of difficult-to-target transcription factors, particularly STAT3, a key molecular hub driving immune suppression. Cytosolic STAT3 dimers that bind decoy SNAs are sequestered outside of the nucleus, preventing their transcriptional activity. The use of bi-SNAs will address the challenge of nucleic acid delivery to intracranial tumor sites and the issue of intracellular stability. Bi-SNAs can exploit the multivalent presentation of ODNs to enhance the formation of cGAS:dsDNA and STAT3:dsDNA complexes. The identification of a unique class of bimodal immunotherapeutics that act as cGAS-STING pathway agonists and STAT3 inhibitors via the robust delivery of ODN payloads may prove instrumental for overcoming immunosuppression in GBM, especially when consideration is given to the spatiotemporally controlled release of optimized SNAs using local hydrogel implants.

2.3. Spatially Controlled Tumor Removal and GBM TME Modulation by FUS and LITT. Minimally invasive surgical technologies, such as stereotactic LITT and FUS, have recently emerged and can not only ablate tumors but also exert additional therapeutically relevant effects on the TME, including immune

cell activation and infiltration. Depending on the parameters utilized—frequency, pressure, duty cycle, and duration—FUS can achieve tissue ablation via hyperthermia or histotripsy (144, 145). While the tissue ablative capability of FUS has been useful for essential tremor and neurodegenerative diseases (145), the ablation of larger tissue areas for patient brain tumor treatments is in its infancy and not yet achievable (146). FUS can enable the passage of molecules across the BBB without significant injury to normal brain when used in conjunction with intravenous microbubbles (147). FUS appears to inhibit the function of tight junctions through either a reduction in component proteins or alterations in subcellular localization or interactions of these proteins (147). There is also evidence that caveolin-1-dependent endothelial cell transcytosis may be stimulated by FUS to deliver large molecules (in the range of a few hundred kDa) from the systemic circulation (148). FUS has been shown to increase brain access of therapeutic antibodies, such as checkpoint-inhibiting antibodies, in preclinical models of GBM (13).

The use of LITT has been described for treating primary and metastatic brain tumors, radiation necrosis, and epilepsy foci (149, 150). During a LITT ablation, photons emitted by the laser optical fiber are absorbed by tumor cell chromophores, resulting in chromophore excitation followed by the release of thermal energy (149, 151). When a sufficiently elevated temperature is achieved, protein denaturation, cellular necrosis, and tissue coagulation occur. A recent multicenter matched cohort study compared outcomes of patients undergoing LITT versus biopsy followed by standard chemo- and radiation therapy to treat newly diagnosed GBM. This study showed that patients undergoing LITT had improved survival compared to matched patients undergoing biopsy alone. Thus, LITT may represent an alternative to needle biopsy in patients with difficult-to-access tumors or who cannot tolerate craniotomy (152). The LAANTERN prospective multicenter registry (NCT02392078) demonstrated that LITT offers an effective cytoreductive approach for both newly diagnosed and recurrent GBM patients. Importantly, its use in newly diagnosed patients followed by post-LITT chemoradiotherapy produced a median overall survival non-inferior to that of patients treated with conventional surgical resection and chemoradiation therapy, thus making LITT a viable alternative in GBM patients, particularly those with tumors not amenable to resection (153).

Human patient data indicates that LITT increases local BBB permeability in high-grade glioma patients for approximately 4 to 6 wk (154). These observations were verified in preclinical models by delivering laser treatment to mouse cortex or orthotopically implanted GBM tumors in syngeneic and human patient-derived xenograft (PDX) models (Fig. 1 C, *i*) (155). MRI demonstrated that mouse brain tumors were targeted in a manner similar to that of human tumors, characterized by a central area of heterogeneous T2W hypointensity (Fig. 1 C, *ii*) (116). LITT treatment significantly reduced tumor burden as assessed by bioluminescence imaging of luciferase-expressing PDX-bearing mice (Fig. 1 C, *iii*). Clinical evidence that this property of LITT might be leveraged for therapeutic benefit comes from a recent phase II clinical trial in recurrent GBM, which suggested that LITT augments the clinical activity of adjuvant low-dose doxorubicin, a drug that typically does not penetrate

the BBB (156). Indeed, laser-treated brain tumors in mice showed substantial infiltration of intravenously delivered human IgG primarily in the laser penumbra, in contrast to minimal IgG infiltration in sham-treated brains (155). These results highlight the possibility of using LITT to enhance brain penetration of systemically delivered treatments, including antibody-based immunotherapies. In preclinical models, the underlying mechanism of augmented BBB permeability was associated with the disruption of brain endothelial cell tight junctions and increased endothelial transcytosis in the laser penumbra (Fig. 1 C, *iv*) (155, 157–159).

Beyond cytoreduction and altering BBB permeability, both laser therapy and FUS may remodel the TME, including immunological changes, with implications for treatment. Hyperthermia in tumors of other organ systems has resulted in the upregulation of specific cytokines, augmentation of antigen presentation, and increased activity of cytotoxic T cells and NK cells (160). Studies in non-glioma tumors indicate that hyperthermia also increases production and release of DAMPs (including heat shock proteins) and tumor-derived exosomes that can potentiate immune responses (96). Preliminary evidence has identified local and systemic immune effects of LITT akin to an *in situ* vaccination approach (161). In patients with recurrent GBM treated with LITT, co-culture experiments of tumor lysate-pulsed dendritic cells with patient PBMCs taken post-LITT showed increased IFN- γ production compared to co-culture with PBMCs collected before LITT, suggesting an adaptive immune response triggered by LITT. Also, FUS treatment can trigger specific immune effects, including enhanced vascular permeability and resultant T cell infiltration into the TME, leading to tumor growth suppression (162).

Collectively, these results credential FUS and LITT as promising anti-neoplastic technologies. Future research should investigate FUS and LITT effects on tumor, immune, and BBB endothelial cells to precisely define technology-induced changes in the GBM TME and determine the precise molecular mechanisms of BBB permeability and drug transcytosis. Finally, ongoing research should define the immunogenic effects of LITT and FUS to develop powerful synergistic immune-oncology strategies.

3. Outlook

Neuro-oncology research must develop novel approaches to achieve meaningful prognostic improvements for GBM patients. These should include the establishment of combinatorial, multimodal, neuro- and nanotechnology-enabled immunotherapies. Establishing these platforms as key modalities for the treatment of GBM will only be possible through massive parallel research activities and collaborations across different fields. These collaborations will be instrumental in addressing potential FDA regulatory hurdles, including the reconciliation of differences between animal models and human disease in the setting of immunotherapy, strategies to ensure good manufacturing practices in the scale-up of NP synthesis, thorough assessment of drug pharmacokinetics, and optimal clinical trial design while advancing innovative treatments that can significantly enhance the prognosis and quality of life for GBM patients.

4. Conclusions

The development of local multimodal, neuro- and nano-technology-enabled immunotherapies, combined with systemic precision immunotherapies, will be an important step forward in the battle against GBM. The importance of spatiotemporally controlled drug release in the GBM TME cannot be overstated. Precise and controlled delivery is essential for training the immune system effectively, allowing it to identify and eliminate cancer cells, create anti-tumor immune memory, and, consequently, extend survival rates. A key factor in this context is the structure of nanotherapeutics, which plays a pivotal role in determining their therapeutic efficacy. Due to their multivalency, nanostructures such as SNAs or multivalent polymer-based NPs have demonstrated greater potential in activating antigen-presenting cells and priming antigen-specific T cells than therapeutics that do not exploit the three-dimensional presentation of immunogenic cues. The modular and chemically well-defined structures of adhesive hydrogels, CDN-NPs, and SNAs provide scope for systematic variation, optimizing TME infiltration, persistent accumulation, and cellular uptake. Rational nanotherapeutic development that reveals relationships between structure and performance is poised to create an inflection point in our understanding of immunotherapies at the nanoscale.

Development of potent, clinical-grade STING agonists provides an opportunity to explore STING activation as a GBM immunotherapeutic modality; the hypothesis that nano-technology-enabled delivery platforms designed for the local, spatiotemporally controlled, and cytosolic release of CDNs will improve stability, reduce off-target effects, increase dose responses, and more robustly promote anti-tumor immune memory must be tested. It is also important to test the hypothesis that targeting the DNA sensing enzyme cGAS upstream of STING with concomitant inhibition of STAT3-driven

immunosuppression will result in robust innate and adaptive immune responses, through the induction of type I interferons and pro-inflammatory cytokines, reprogramming the immunosuppressive myeloid cells and promoting T cell recruitment and activation.

Little is known about the biological effects of both LITT and FUS on the GBM TME beyond thermal effects on tumor cell death and whether these effects might be harnessed for therapeutic benefits. Human and preclinical studies demonstrated that these device technologies increase local BBB permeability for a prolonged period, enable the therapeutic delivery of chemotherapy and biotherapeutic antibodies to prolong survival as seen in animal models and in GBM patients, and potentially reprogram the tumor-associated immune system. The use of in vivo model systems in combination with the modifiable parameters of LITT and FUS can be optimized to enhance TME remodeling to synergize with locally and systemically administered immunotherapies in preclinical GBM models.

Data, Materials, and Software Availability. Previously published data were used for this work (all citations are included in the Main Text, see figure captions).

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Correction

Medical Sciences, Chemistry

Correction for “Multimodal neuro-nanotechnology: Challenging the existing paradigm in glioblastoma therapy,” by Sergej Kudruk, Connor M. Forsyth, Michelle Z. Dion, Jenny K. Hedlund Orbeck, Jingqin Luo, Robyn S. Klein, Albert H. Kim, Amy B. Heimberger, Chad A. Mirkin, Alexander H. Stegh, and Natalie Artzi, which published February 12, 2024; 10.1073/pnas.2306973121 (*Proc. Natl. Acad. Sci. U.S.A.* **121**, e2306973121).

Upon request, the four additional references below have been added to the article.

130. W. L. Daniel, U. Lorch, S. Mix, A. S. Bexon, A first-in-human phase I study of cavrotolimod, a TLR9 agonist spherical nucleic acid, in healthy participants: Evidence of immune activation. *Front Immunol.* **13**, 1073777 (2022).

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A citation to the first new reference should be included on page 6, right column, first paragraph, line 7, where the reference callout should appear as “(130).”

Citations to the remaining three new references should be included on page 6, right column, first paragraph, line 12, where the reference callout should appear as “(131–133).”

In addition, the legend and in-text citation to Fig. 1 were accidentally misnumbered. The figure and its corrected legend appear below. The online version has been corrected.

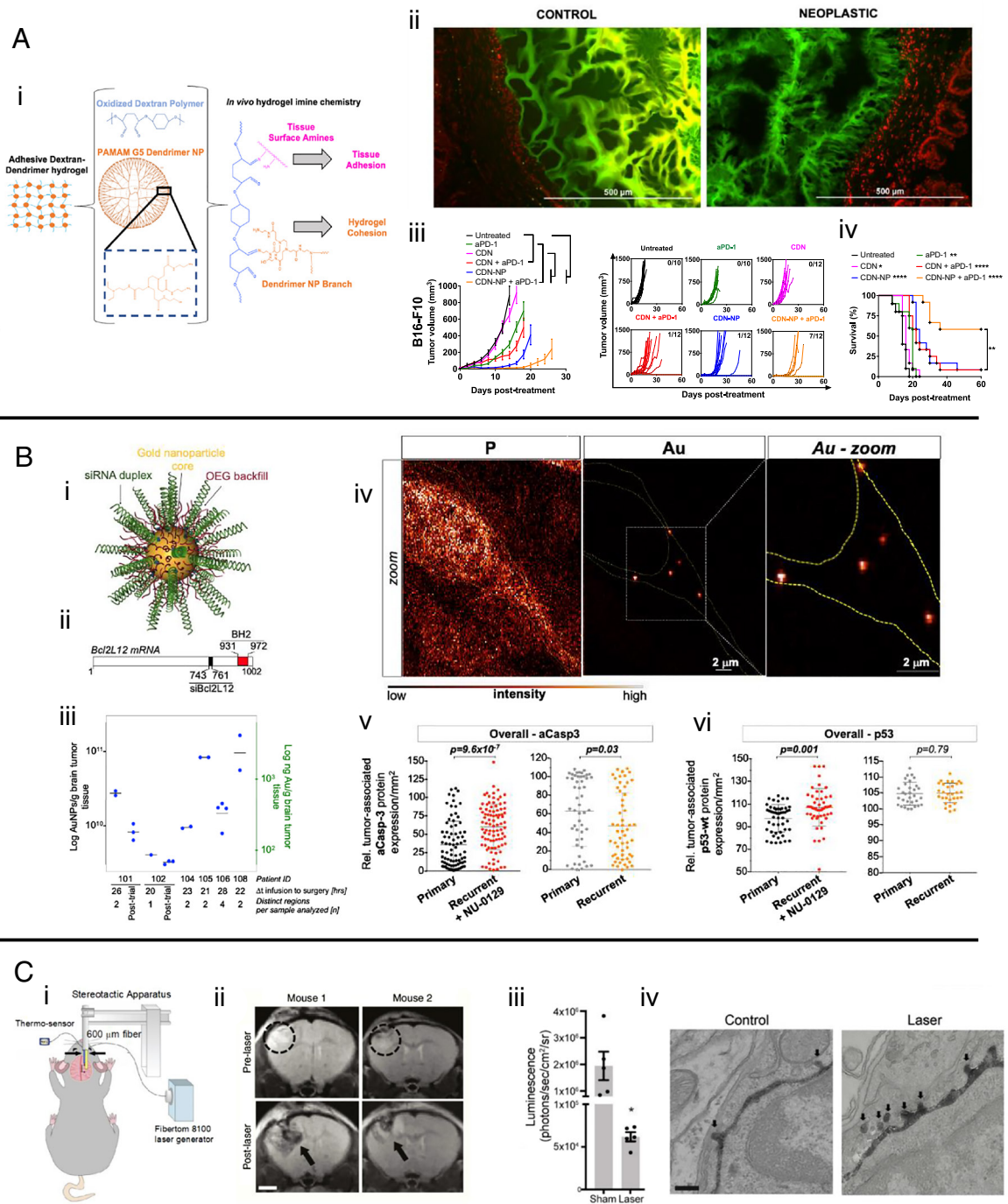


Fig. 1. (A) Potential of locally administered adhesive hydrogels combined with immunostimulatory NPs for anticancer therapy. (i) Schematic showing the chemical structure of adhesive hydrogels formed from dendrimer nanoparticles and oxidized dextran and their beneficial *in vivo* properties. (ii) Confocal images showing tissue (red) interactions of implanted hydrogels (green) in healthy and neoplastic tissue. (iii) Tumor volume and percent survival of B16-tumor bearing mice treated with CDN-NPs or CDN alone in combination with anti-PD-1 antibodies (iii, iv) reproduced from ref. 94. (B) Gene regulatory SNAs as an emerging therapeutic modality for GBM. (i) Schematic of the modular SNA architecture (NU-0129). (ii) *Bcl2L12* mRNA with binding site for the siRNA oligonucleotide used to functionalize gold nanoparticle cores (nucleotide position 743 to 761; black box). Positions for nucleotides encoding the C-terminal BH2 are indicated (nucleotide position 931 to 972; red box). (iii) ICP-MS analysis of bulk patient GBM tissue, including tumor recurrences post NU-0129 trial enrollment for patients 101 and 102; tumors recurred 159 and 174 days post NU-109 trial enrollment, respectively. N, number of tumor regions sampled. Δt , time from surgery to infusion. Shown is the median. (iv) XFM-Bionanoprobe assessment of patient tumor reveals extranuclear/cytoplasmic distribution of Au in tumor cells. (v, vi) Protein expression of active caspase-3 and wild-type p53 in matched newly diagnosed and NU-0129-treated recurrent GBM. Quantification of overall IHC staining intensities for the *Bcl2L12* downstream effectors active caspase-3 and wild-type p53. From ref. 129. Reprinted with permission from AAAS. (C) LITT productively modulates the BBB in models of GBM. (i) Schematic of murine LITT administration. (ii) T2-weighted MR images of LITT-treated tumor-bearing mice. (iii) Quantification of tumor volume using bioluminescence imaging post-treatment. (iv) Representative transmission electron microscopy images following intravenous HRP administration in the sham and laser-treated brain (post-laser day 3). Arrows indicate HRP-filled vesicles within endothelial cells. (Scale bar, 100 nm.) Reproduced from ref. 155, Oxford University Press.

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