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Precision Medicine in Pediatric Neurooncology: A Review

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Abstract

Central nervous system tumors are the leading cause of cancer related death in children. Despite much progress in the field of pediatric neurooncology, modern combination treatment regimens often result in significant late effects, such as neurocognitive deficits, endocrine dysfunction, secondary malignancies, and a host of other chronic health problems. Precision medicine strategies applied to pediatric neurooncology target specific characteristics of individual patients' tumors to

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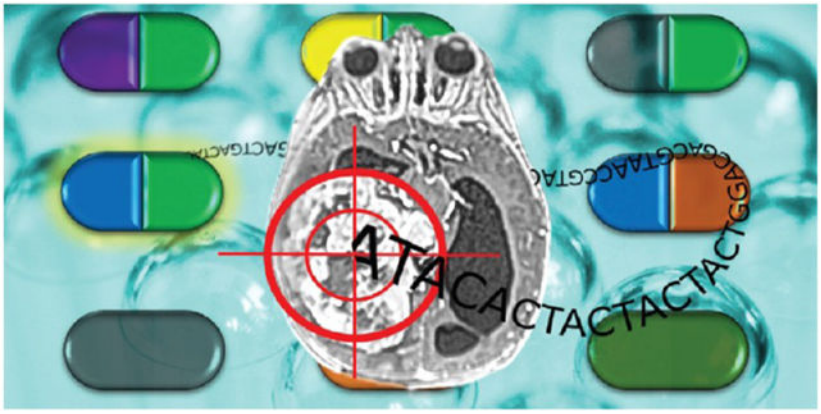
achieve maximal killing of neoplastic cells while minimizing unwanted adverse effects. Here, we review emerging trends and the current literature that have guided the development of new molecularly based classification schemas, promising diagnostic techniques, targeted therapies, and delivery platforms for the treatment of pediatric central nervous system tumors.

Graphical Abstract



Keywords

Precision medicine; pediatrics; neurooncology; review

Neoplasms of the central nervous system (CNS) constitute the majority of solid tumor diagnoses and remain the leading oncologic cause of mortality in children.[1, 2] Aggressive multimodal treatments have yielded demonstrable improvements in survival in some cases.[3] However, the particular susceptibility of the pediatric population to the long-term effects of these treatments is well documented, necessitating refinement of treatment protocols.[4, 5] Late effects of existing chemotherapies and photon irradiation in young children include cognitive decline, endocrine dysfunction, secondary malignancy, vasculopathy, and other factors that impact quality of life significantly.[6-8] Further, these outcomes may be progressive and irreversible, especially in patients aged 5 years or younger.[4]

The importance of tailoring therapies to address the requirements of the developing pediatric patient is a unique challenge. As it is recognized that CNS neoplasms found primarily in children harbor molecular and genetic signatures vastly different from those found in adult patients, the application of precision medicine in the diagnosis and management of pediatric CNS malignancies has received increased attention.[9] In this Review, we highlight the impact that advances in precision medicine are making within pediatric neurooncology to drive progress in the development of innovative imaging and molecular technologies that achieve improved detection and targeting of CNS tumors. Insights gained from these efforts have motivated the updated World Health Organization (WHO) classification system for CNS tumors and led to the expanded role of next-generation profiling techniques to address heterogeneity and variability between tumors of a particular subgroup. In turn, these data are applied increasingly to guide the design of new targeted treatments, including emerging molecular, radiation, and immunotherapeutic strategies. Finally, to stimulate broader

interactions between experts in the physical and neurosciences with clinician colleagues, we review nanoscale platforms such as polymeric nanoparticles (PNPs), liposomes, nanoparticle albumin-bound technology, and molecular targeted nano-particles, which are currently being explored as therapeutic options for CNS tumors.[10-18]

CLASSIFICATION

The revised 2016 WHO system of classification for CNS neoplasms reflected a dramatic change in the characterization of CNS tumors. For the first time, molecular parameters were incorporated into a system that previously relied entirely on histology, demonstrating the capability for increasingly precise categorization availed by modern diagnostic techniques.[19] The three most common categories of pediatric CNS malignancies are embryonal tumors, which are comprised primarily of medulloblastoma, gliomas, and ependymal tumors (Figure 1).[20] Of these, gliomas and medulloblastomas underwent major restructuring under the new WHO classification system, whereby all three incorporated genetically defined entities that are highlighted below. It is hoped that the 2016 CNS WHO system will improve diagnostic accuracy, leading to more precise therapeutic planning (which will be detailed in a later section) and, ultimately, better outcomes for patients with brain tumors.

Medulloblastomas.

Medulloblastomas are currently divided into four distinct histologic and molecular subgroups: Wnt, Sonic Hedgehog (SHH), group 3, and group 4;[21, 22] however, the clinical behavior of these malignancies remains heterogeneous. Integrated analyses have defined clinically significant subtypes within these subgroups, which may enable improved risk stratification over existing schema and more tailored treatment decisions.[23] As an example, infant medulloblastoma is currently partitioned into two groups by histologic findings; however, there appears to be no difference in prognosis between the two strata. By combining gene expression with DNA methylation data, Cavalli et al. identified four infant SHH molecular subtypes with significant differences in survival.[24] Two of these subtypes appear to be extremely low risk and may benefit from de-escalation of therapy in future clinical trials, sparing them from the adverse effects of more aggressive treatment regimens. These findings indicate that more in-depth studies directed at unraveling the heterogeneities observed within the medulloblastoma landscape are needed to establish more precise stratification schemes that better define risk and enable patient-specific guidance.

Gliomas.

Pediatric high-grade gliomas (HGGs) are composed primarily of anaplastic astrocytomas and glioblastoma and are associated with particularly poor prognoses, with 5-year survival rates of less than 20%. The 2016 CNS WHO classification system incorporated molecular variants of high-grade gliomas, including IDH-wildtype and IDH mutant glioblastoma, and H3.3K27M mutant diffuse midline glioma (which includes tumors previously referred to as diffuse intrinsic pontine glioma, DIPG).[19] Due to their relative rarity, pediatric HGGs have traditionally been treated based on proven adult regimens, with minimal clinical benefit.[25, 26] Researchers have found that while phenotypically indistinguishable, pediatric HGGs manifest characteristic genetic alterations distinct from those seen in adult HGGs.[27] Next-

generation sequencing has revealed that somatic mutations in genes encoding histones are characteristic of pediatric high-grade gliomas, specifically H3F3A (replication-independent histone 3 variant H3.3) as well as in histone 3.1, whereas IDH mutant gliomas are extremely rare.[28] In addition, ATRX (α-thalassemia/mental retardation syndrome X-linked) and DAXX (death-domain associated protein) in combination with TP53 mutations are frequently seen in pediatric HGGs.[28, 29] Furthermore, approximately 80% of pediatric GBMs demonstrate activation of the PI3K/Akt/mTOR pathway;[30] mutations in epidermal growth factor receptor (EGFR) [31] and platelet-derived growth factor receptor (PDGFR) [27, 32] have also been associated. Thus, newer molecular diagnostic techniques make it evident that histopathologically identical entities such as pediatric HGG may harbor profoundly different, potentially targetable underlying mechanisms.

Diffuse midline glioma is a malignant, infiltrative glial neoplasm of the ventral pons associated with a dismal outcome. Patients present with rapid onset of symptoms, and the median survival is 9–12 months. Most patients do not live 2 years past diagnosis and chemotherapy has been ineffective. Radiation is considered the standard of care and is utilized for extension of the symptom-free period but no therapy has yet significantly changed overall outcome. Recent innovations in biopsy of the pons have resulted in fascinating new molecular findings in this disease. Investigators have identified that 80% of diffuse midline glioma cases harbor histone 3.3 or 3.1 mutations, most frequently H3.3K27M.[33] These mutations result in hypomethylation of H3 proteins and alter epigenetic regulation of genes crucial for cell cycle function and oncogenesis. These histone mutations also co-occur predictably with other mutations.[33-36] For example, H3.1 mutations co-occur with ACVR1 mutations most commonly while H3.3 mutations co-occur with p53 and PDGFRA mutations. Other accessory driver mutations have been identified including mutations in PIK3R1 and PIK3CA. H3.3K27M mutations, independent of histopathological features, are universally associated with poor survival outcomes in diffuse midline glioma; in fact, tumors possessing these anamolies were deemed a distinct entity in the 2016 WHO classification system. Researchers are searching for and studying targeted therapies actively.

Low-grade gliomas (LGGs), defined in the 2007 WHO CNS classification system as grades I and II based on histological criteria,[37] comprise the most common type of pediatric CNS tumor.[20] A heterogeneous group, LGGs consist of oligodendroglioma, pilocytic astrocytoma, subependymal giant cell astrocytoma, angiocentric glioma, and others. As a whole, pediatric LGGs are associated with excellent long-term survival compared to adults. [38, 39] While many historical studies treated LGGs as a single cohort, advances in molecular characterization techniques bolster support for individualized stratification to minimize long-term treatment-related morbidities. For instance, neurofibromatosis type 1, a genetic syndrome caused by a mutation in the neurofibromin 1 gene, is associated with pilocytic astrocytoma and diffusely infiltrating astrocytoma[40] and a number of other malignancies that may be amenable to biologically targeted treatments.[41] Pilocytic astrocytomas have been found to harbor mutations in BRAF, neurotrophic tyrosine kinase type 2 (NTRK2), and histone H3; all of which are being specifically targeted in current pediatric clinical trials. [42, 43]

Ependymal Tumors.

Ependymoma is the third most common pediatric brain tumor type, and is frequently associated with poor long-term survival outcomes.[44] The 2016 WHO classification system divides ependymal tumors into subependymoma, myxopapillary ependymoma; ependymoma; anaplastic ependymoma; and ependymoma, RELA fusion-positive. Ependymomas that have undergone chromo-thripsis (identified by the C11orf95-RELA fusion) were the lone genetically defined subtype accepted in the updated WHO criteria. The authors acknowledge that this classification system is imperfect and of little prognostic benefit, citing the need for more reproducible data before further changes can be made. The standard of care for ependymoma is maximal safe resection followed by focal radiation therapy. However, some studies suggest that in a subset of patients with ependymoma, surgery alone without radiation or chemotherapy may suffice.[45] Emerging classification schemes based on methylation profiling data[46] yield improved prognostic significance relative to conventional histologic grading and will be important to incorporate into future preclinical models and clinical trials targeting these pediatric CNS malignancies.[47]

IMAGING

Concern for radiation exposure in developing children continues to drive the integration of imaging and radiation therapy to make treatment of pediatric brain malignancies safer and more targeted. Several diagnostic imaging techniques have been reported that aid in the classification and prediction of outcomes in pediatric brain tumors. For example, advanced magnetic resonance imaging (MRI) techniques such as diffusion tensor imaging (DTI) and dynamic susceptibility-weighted contrast-enhanced (DSC) perfusion have proven useful in glial tumors, particularly in their surgical management.[48-51] Positron emission tomography (PET) is an imaging modality that enables noninvasive quantitation of biochemical processes such as glucose metabolism and oncogenic drivers such as receptor tyrosine kinases.[52] The development of novel radiotracers for use in combination with PET improves delineation of active tissue within heterogeneous CNS tumors such as gliomas. By applying PET tracers specifically targeting factors linked to glioma-associated inflammation, such as the translocator protein (TSPO) and matrix metalloproteinases (MMP), Zinnhardt et al. characterized the tumor micro-environment in a murine model of human glioma non-invasively, which matched histological findings.[53] This method provided information that could not be detected by a single tracer and/or MRI alone, informing similar PET-based diagnostic approaches to characterize pediatric CNS malignancies.

Another perfusion-imaging technique, arterial spin labeling (ASL), represents an intriguiging alternative to MRI-based approaches such as DSC. In ASL, radiofrequency pulses are used to label endogenous protons within the arterial blood in order to measure cerebral blood flow, circumventing the need for intravenous gadolinium contrast agents, such as those required for DSC. In rare cases where the use of intravenous infusions of contrast agents may be contraindicated in the pediatric population (e.g., nephrogenic systemic fibrosis associated with acute and/or chronic renal impairment), ASL may provide similar data that may be useful in guiding clinical decision making for these patients.[54]

PROMISING TUMOR PROFILING TECHNIQUES

In this section, novel molecular profiling techniques with the potential to migrate rapidly from preclinical settings into common clinical practice are discussed. These technologies are quickly reaching Clinical Laboratory Improvement Amendment (CLIA) approved status and impacting patient care. Note that the clinical utility of molecular biomarkers has already been established for a variety of cancers, including glioma, and specific well-established assays have had their analytical validity demonstrated for detection of those mutations in patient samples.[55] Markers for glioma include: 1p/19q codeletion, analyzed by fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and multiplex ligation-dependent probe amplification (MPLA);[56] IDH mutation established by immunohistochemistry (IHC), and DNA sequencing;[57, 58] and MGMT methylation identified by methylation-specific (MS)-PCR, MS-pyrosequencing, and MS-MPLA.[59]

Molecular classification has also impacted the understanding of CNS primitive neuroectodermal tumors (CNS-PNETs), aggressive embryonal tumors occurring mostly in the pediatric population. These CNS malignancies often present challenges in neuropathological diagnosis due to a lack of defining molecular markers and histological overlap with other high-grade neuroepithelial tumors. Five subcategories of CNS-PNETs had been described by the WHO based on morphological features but more recent studies indicated that CNS-PNETs are molecularly heterogeneous, pointing to the need to classify this group better. To address this issue, Sturm et al. analyzed genome-wide DNA methylation profiles of tumors that had been institutionally classified as CNS-PNETs and found that over half of the samples did not form a distinct cluster, but rather displayed molecular profiles indistinguishable from other well-defined CNS tumor entities. From the remainder of the original CNS-PNETs, four new classifications were suggested based on genetic, histopathological, and clinical features of the tumor.[60] Additionally, Johann et al. recently identified that atypical teratoid/rhabdoid tumors (AT/RTs), another group of pediatric CNS malignancy with poor prognoses, are comprised of three distinct epigenetic subgroups, with distinct clinical characteristics and regulatory networks that can potentially be targeted therapeutically.[61] These studies reinforce the expanding importance of considering molecular profiling data when assigning a diagnosis to a CNS neoplasm given that this decision directs the selection of appropriate therapies to target aggressive pediatric brain cancers.

To address the issue of intratumoral heterogeneity in designing a treatment or assigning prognosis upon diagnosis, single-cell RNA-seq has been implemented as a useful technique to understand the tumor ecosystem and the diversity of cells therein.[62] Patel et al. reported using the previously developed Smart-seq technique to profile 430 cells from five different primary glioblastomas, obtaining single cell full-length transcriptomes.[63] Their results corroborate the idea that these tumors present variable expression of diverse transcriptional programs with regards to oncogenic signaling, proliferation, complement/immune response, and hypoxia. They also show that different glioblastoma subtype classifiers are found within different cells of a single tumor, an observation that may be critical for prognosis and choice of treatment protocols.[64]

To address the challenges faced by low-input samples when using the aforementioned techniques, a portable, low-cost platform called Seq-Well has been developed by Gierahn et al. that enables high throughput, parallel single-cell RNA sequencing.[65] This microwell-based platform utilizes barcoded mRNA (mRNA) capture beads while single cells are sealed in subnanoliter wells by a semipermeable membrane that permits efficient cell lysis and transcript capture (Figure 2). Similar microfluidic approaches for single-cell multiplex processing are being developed, such as valve- and droplet-based platforms.[66] Although the array of applications for these emerging techniques is vast, one may envision that they could be applied to a variety of cancer cells to understand tumor heterogeneity for the benefit of patient care.

While these increasingly precise technologies transition to the clinic, assessing genomic and epigenomic modifications that may have important implications in cancer biology still requires days to weeks for sample processing. Eukirchen et al. have validated the utility of a pocket-sized nanopore sequencing device that is capable of detecting copy number variants, point mutations, and methylation profiling within just 1 day for the analysis of brain cancer biopsies.[67] Nanopore sequencing evaluates and interprets changes in ionic currents observed when single DNA or RNA molecules pass through a nanometer-size protein pore that can discriminate between nucleotides.[68, 69] This platform can generate copy number variation data as well as detect single-base modifications, such as methylation profiles,[70] concurrently in a single sequencing run. Even though this method has lower throughput than other technologies, it can yield ~0.1× genome coverage within 6 h and it is unique in that it enables low-cost, real-time reads outside of a laboratory setting. Furthermore, this platform was able to distinguish gliomas, medulloblastomas, and brain metastases of different primary sites from patient brain tumor biopsy samples, with copy number and epigenetic profiles that correlated well with matched microarray data.[67]

Even though many of the technologies discussed have not been applied widely to pediatric CNS malignancies, Ramkissoon et al. confirmed that a multiplexed targeted exome-based sequencing (OncoPanel) in combination with a clinical genome-wide aCGH assay (OncoCopy) can provide critical information for the treatment of pediatric brain tumors, by alerting pediatric oncologists to potential clinically relevant targets.[71] These CLIA-certified platforms are a promising example of the clinical utility and validity of these emerging technologies.[72] A novel genetic algorithm-based random forest modeling technique has also been developed that enables reduction in the complexity of large gene disease signatures.[73] This method has been explored for glioblastoma multiforme (GBM) samples, but it could potentially applied to other CNS neoplasms in order to facilitate interpretation of the increasing body of nucleotide-level information about these cancers. Other novel, potentially applicable methods of individualized analysis include the utilization of CRISPR-Cas9 to conduct large-scale genetic screening[74] and patient-derived xenografts, which enable tumor profiling and drug screening.[75] Some recent studies have translated pediatric tumor gemonic data to the clinic and were successful in identifying actionable findings that guided treatment approaches in a substantial proportion of the studied population, including in solid CNS tumors.[76-78]

Finally, tumor-associated short noncoding RNAs found in CSF or blood are also thought to provide important insight into brain tumor biology due to the differences found between expression profiles in healthy individuals and patients (Figure 3). The aforementioned next-generation sequencing platforms could be applied to analyze and to determine their utility in the diagnosis of pediatric brain malignancies and in disease surveillance.[79]

RADIATION THERAPY

Radiation therapy has been used for years in the treatment of childhood brain tumors and continues to be a mainstay in the management of some pediatric CNS malignancies. However, patients subjected to high doses of radiation are at increased risk of neurocognitive damage, growth arrest, damage to the cerebral vasculature and endocrine glands, inner ear dysfunction, as well as development of secondary cancers. Many efforts are therefore aimed at improving the precision and efficacy of radiation therapy treatments while reducing risks.[80, 81] One such modality is proton beam therapy, which is an alternative to photons to deliver therapeutic radiation to treat CNS tumors.[82, 83]

Both proton and photon radiotherapy function by depositing energy in cells, which causes damage to the cellular DNA through the formation of free radicals; when DNA damage is not repaired, the cell dies. Tumor cells are known for having decreased capacity for DNA damage repair, which makes them more susceptible to damage and death through this method. However, normal cells are not entirely spared in the process and thus are also subjected to short- and long-term toxicity; therefore, reducing exposure of normal tissue to radiation is critically important (Figure 4).[84]

Proton therapy has only recently been used in pediatric cases, demonstrating equivalent efficacy with the potential for reduced side effects.[85] The key is that the properties of proton beams allow decreased doses to normal tissues surrounding the tumor when compared to conventional photon therapy.[86] Trials using this technique have shown mixed results in terms of local control, progression-free survival and avoidance of decreased IQ or overall adaptive skills.[87-92] This therapy has been used for CNS germ cell tumors,[88] localized ependymomas,[89] low-grade gliomas,[90] medulloblastomas,[91] chordomas,[93] and craniopharyngiomas.[93]

Other charged particle therapy technologies being developed utilize neutrons and carbon ions, but have not yet been implemented for pediatric tumors. Image-guided radiation therapy has also become increasingly common, and provides great improvements in radiotherapy accuracy. These techniques utilize high-quality images to guide target visualization with millimeter precision. Heightened precision enables reduction in the area of healthy tissue affected by therapy.[84, 94]

Nanoparticles have been developed in recent years that can work synergistically with radiation therapy to improve outcomes for brain tumor treatment. One of these approaches is boron neutron capture therapy, which relies on selectively concentrating boron compounds in tumor cells and then subjecting them to epithermal neutron beam radiation, thus depositing a large dose gradient between tumor cells and normal cells.[95] Many other groups have also explored the use of gold nanoparticles to radiosensitize tumor cells. These efforts

have shown promising results in murine animal models of various brain tumors and include gold nanoparticles on their own,[96, 97] coated with gadolinium chelates,[98] pH-sensitive tumor-targetting peptides [99] and even in conjunction with superparamagnetic iron oxide in micelles;[100] one study reports that silver nanoparticles can outperform gold nanoparticles for in vitro radiosensitization of glioma cells.[101] Other groups have been successful in sensitizing brain tumor cells using nanoparticles that silence genome components. For instance, Zhang's group has conducted nanoparticle-mediated siRNA knockdown of the DNA repair enzyme apurinic endonuclease 1 (Ape1) in a murine model of glioblastoma [102] and in pediatric ependymoma and medulloblastoma cells in vitro, reporting improvements in circumventing radiation resistance in these tumors.[103, 104]

MOLECULAR THERAPY

Whereas conventional chemotherapy indiscriminately targets rapidly dividing cells, leading to a host of adverse effects, researchers are now seeking to exploit tumor-specific molecular pathways. One of the major challenges in pediatric neurooncology today is the paucity of efficacious treatments that target the growing list of molecular aberrations identified by increasingly sophisticated genomic and epigenomic technologies.[105] Other treatments, while promising in their scientific rationale, have not demonstrated clinical benefit. Here, we briefly review a selection of targeted agents for some of the previously outlined tumors; the discussed, currently open clinical trials for pediatric brain tumors are listed in Table 1.

Embryonal Tumors.

For instance, a phase II trial evaluated vismodegib, a first-in-class smoothened receptor antagonist targeting the SHH pathway, demonstrating safety and possible activity in pediatric patients with recurrent SHH subtype medulloblastoma.[106] The phase II SJMB12 trial is currently stratifying patients with medulloblastoma based on clinical risk and molecular subtype, evaluating whether patients with low-risk Wnt tumors can be treated with lower doses of radiation and chemotherapy without impacting survival.[107] TB-403, a monoclonal antibody against placental growth factor that showed activity in a murine medulloblastoma model,[108] is currently being evaluated in a phase 1 and phase 2 trial in pediatric patients with relapsed or refractory medulloblastoma ().

Atypical teratoid rhabdoid tumor (AT/RT), a rare pediatric CNS embryonal tumor defined by biallelic inactivation of the *INI-1* locus,[109] is associated with a grim prognosis despite multimodal therapy.[110] An ongoing phase I trial () is currently evaluating the effects of tazemetostat (an EZH2 inhibitor) in patients with relapsed or refractory INI1-negative malignancies, which include AT/RT. A phase II trial () is studying the effects of the auroa A kinase inhibitor alisertib in pediatric patients with AT/RT.

Gliomas.

Gene expression profiles of gliomas have been analyzed, searching for potentially exploitable driver mutations as the number of clinical trials investigating targeted therapies continues its rapid expansion.[111, 112] As previously mentioned, sequencing of pediatric gliomas have identified mutations in the genes encoding histones 3.1 and 3.3, as well as

chromatin modifiers ATRX and DAXX. Histones play an integral part in the packaging of DNA within cells, regulating its expression at the epigenetic level.[113] Given their frequency, researchers are attempting to utilize these mutations as targets for therapy. For example, a multihistone deacetylase inhibitor, panobinostat, subsequently showed therapeutic efficacy in H3.3K27M mutant diffuse midline glioma cell culture and xenograft models,[114] and is currently undergoing a phase I trial in children (). Another phase I trial is evaluating H3.3K27M peptide vaccine conjugated with tetanus toxoid () in pediatric patients with diffuse midline glioma.

Other studies include a recently completed phase I trial of crenolanib (), a PDGFR inhibitor, in patients with recurrent, high-grade glioma and diffuse midline glioma and a molecular profiling individualized treatment plan trial for diffuse midline glioma (). Hopefully, these more targeted and personalized medicine approaches will result in improved outcome for these patients in the near future.

Epithelial growth factor receptor (EGFR) is overexpressed in a subset of astrocytic pediatric gliomas,[115] and can be targeted by agents such as erlotinib, which demonstrated tolerability in a phase I study in pediatric patients with brainstem glioma,[116] but little effect in phase II.[117] Multiple studies have evaluated a host of other agents with promise, but found little clinical benefit, including tipifarnib, a farnesyltransferase inhibitor, was studied in children with newly diagnosed diffuse midline gliomas but demonstrated no clinical advantage.[118] Enzastaurin, a protein kinase Cβ/PI3K/Akt pathway inhibitor, which showed some promise in a phase I pediatric trial.[119]

A frequent mutation identified in pediatric low-grade gliomas (LGG) involves BRAF, a gene that encodes a crucial enzyme involved in cell survival and growth signaling.[30] Case reports have suggested some efficacy of vemurafenib against $BRAF^{V600E}$ mutant LGG in children;[120, 121] an early phase I trial is currently underway (). Promising results have also emerged from a phase I study of selumetinib, a MEK1 inhibitor, in pediatric patients with recurrent or refractory LGG.[122] In vitro and in vivo model systems of BRAF mutant LGG lend support to combination regimens such as PLX4720 (a BRAF inhibitor) plus selumetinib, or mTOR with MEK blockade.[123, 124] These findings in part motivate a currently open clinical trial exploring the therapeutic role for combination MEK and BRAF inhibition for pediatric HGGs ().

Tuberous sclerosis, a genetic disorder that affects multiple organ systems, leads to overactivation of the mammalian target of rapamycin (mTOR) signaling cascade, can result in subependymal giant cell astrocytoma with biallelic mTOR dysregulation.[125] Everolimus, an mTOR inhibitor, demonstrated significant, sustained reductions in subependymal giant cell astrocytoma volumes in a phase III trial of patients with tuberous sclerosis.[126-128]

Ependymal Tumors.

More than 75% of pediatric ependymomas coexpress ERBB2 and ERBB4;[129] overexpression of VEGF has been associated with poor survival.[130] A phase II trial combined lapatinib, a selective ERBB1 and ERBB2 inhibitor, and bevacizumab in children with recurrent/refractory ependymoma, demonstrating tolerability, but no effect. However,

the authors note that bevacizumab monotherapy has not been shown to be efficacious in pediatric cases of recurrent ependymoma and that intratumoral lapatinib concentrations were below the threshold needed to inhibit the epidermal growth factor receptor (EGFR) and ERBB receptors.[131] Sequencing of pediatric, poor-prognosis posterior fossa ependymomas revealed a CpG island methylator phenotype that has demonstrated in vivo responsiveness to DNA and H3K27 methylation-targeting.[132]

Pediatric MATCH.

In July 2017, The National Cancer Institute (NCI) and the Children's Oncology Group (COG) announced the opening of enrollment for a phase II precision medicine clinical trial, the NCI-COG Pediatric Molecular Analysis for Therapy Choice screening protocol (Pediatric MATCH,). This trial will provide genetic testing for children with various types of tumors, including CNS neoplasms. Patients with mutations that may benefit from one of the more than eight targeted study drugs will be identified for potential directed therapy. Treatment arms that are currently enrolling include larotrectinib (a pan-TRK inhibitor), LY3023414 (a small-molecule inhibitor of class I PI3K isoforms), mTOR, and DNA-PK, plus six other arms. This strategy represents a novel shift in focus from blanket therapy for each disease to a focus on the particular molecular pathways specific to each patient's case.[133]

IMMUNOTHERAPY

Emerging therapies, including immune-targeted strategies, for CNS malignancies have mostly risen from the study of glioblastoma. Cancer immunotherapy encompasses a variety of approaches, with the potential to harness the specificity of adaptive immunity, mediated by T-cells and antibodies, as well as the diverse cytotoxic mechanisms of innate immunity. Immunotherapy strategies include active antitumor vaccination, adoptive transfer activated cytotoxic cells, and blockage of immune inhibitory checkpoints. Preclinical studies, as well as early clinical failures, stress the importance of a multimodal, combinatorial approach to integrating immunotherapy into cancer treatment. Unlike conventional cancer therapies, active immunotherapies hold the potential to induce immune memory.

Dendritic cells (DC) are the most potent antigen-presenting cells in the human immune system; as such, a number of studies have utilized dendritic cell-based immunotherapy in varied cancer types, including for CNS neoplasms. Three studies have evaluated its use in children with brain tumors, finding them to be safe and tolerable in pediatric patients; larger studies are needed to elucidate treatment effect.[134-136] Another approach is to use engineered materials to deliver immune modulating molecules to tumors, cancer vaccines or host immune cells, one avenue being to induce DC activation and subsequent priming of cancer-specific T-cell responses.[137, 138]

Patients' own T-cells can be engineered to seek out and to destroy tumor cells by attaching receptors with affinity to antigens specific to the cancer of interest. These chimeric antigen receptor (CAR) T-cells have demonstrated efficacy in hematologic malignancies such as acute lymphoblastic leukemia.[139] CAR T-cell therapy has proven to be a more difficult venture in solid tumors and by extension, CNS neoplasms. In treating patients with

glioblastoma, researchers have attempted engineering CAR T-cells to target the tumor-associated antigen interleukin-13 receptor alpha 2;[140] epidermal growth factor receptor variant III-specific CAR T-cells have demonstrated efficacy in a murine model.[141] Two major hurdles in utilizing CAR T-cell therapy for CNS tumors are trafficking and persistence:[142] intratumoral T-cell delivery may require repeated intracranial injections, which may not be feasible in a child with a brainstem tumor. Attempts have been made to genetically modify CAR T-cells to respond more effectively to trafficking signals;[143] however, there is a significant need for improved delivery systems, particularly in a space as difficult to access as the CNS. One innovative platform that may be ported to pediatric CNS neoplasms is the CIVO microdosing system, which can precisely inject multiple standard-of-care chemotherapy agents into cancerous lymph nodes (). Notably, CAR T-cells tend to lack long-term survival in the solid tumor microenvironment, often reaching a premature state of exhaustion due to lack of resources for energy production and a more mature phenotype.[144] One innovative engineering approach has been the development of artificial thymic organoids, which may enable the production of younger, more naïve, and efficacious T-cells for use in immunotherapy.[145]

Gene expression data have recently been utilized to identify patients with intratumoral cytokine profiles that may predict more robust responses to pembrolizumab, a PD-1 monoclonal antibody that blocks a major pathway of tumor immune evasion, enabling patients' own immune systems to eliminate cancerous cells more effectively.[146] Furthermore, studies have demonstrated that tumors with defects in mismatch repair pathways (and consequent accumulation of hundreds to thousands of somatic mutations) are more responsive to PD-1 blockade.[147] This finding has been recapitulated in a study wherein two pediatric patients with recurrent, multifocal, biallelic mismatch repair deficient GBMs exhibited sustained responses to pembrolizumab.[148]

Woensel and colleagues designed siRNA targeting Galectin-1 loaded chitosan nanoparticles to silence Gal-1 in the tumor microenvironment. Gal-1 is overexpressed in GBM and drives chemo- and immunotherapy resistance. Intranasal delivery of these particles seemed to promote a switch in the tumor microenvironment composition with respect to myeloid and T-cells, as well as promote normalization of tumor vasculature and increased survival in the animal mouse model. Furthermore, combination of the particles with Temozolomide or immunotherapy (dendritic cell vaccination and PD-1 blocking) showed synergistic effects to improve mice survival outcomes.[149] Vaccination therapies composed of peptides against glioma-associated antigens, which were identified to be overexpressed in LGGs, have also shown promise in children with recurrent LGGs.[150] Myriad studies are currently evaluating various combinations of other immunotherapeutical approaches (e.g., ,).

NANOPARTICLES

Nanoparticle drug delivery platforms have been described in the literature as typically belonging to one of the following categories: liposomes, nanoparticle albumin-bound technology, polymeric nanoparticles (PNPs), and molecular targeted nanoparticles.[11, 151, 152] More recently, drug-encapsulated PNPs have showed promise in targeting aggressive pancreatic ductal adenocarcinoma cells.[153] Nanoparticles targeting various cancers are

continuously being developed and have been described to accumulate preferentially in tumors due to the so-called enhanced permeability and retention (EPR) effect. This favorable effect is attributed to defective vascularization and reduced lymphatic drainage in the tumor microenvironment,[154, 155] although it does not necessarily correlate with improvement in tumor cell uptake of these nanoparticles. Therefore, an employed strategy has been to modify the surface of PNPs for tumor-specific recognition and internalization.

Within the context of GBM, DNA aptamer probes have been selected in vitro that are able to bind to a variety of glioblastoma cells lines with dissociation constants in the nanomolar range, while showing little affinity for other cancer cells. Aptamers are short artificial, single-stranded oligonucleotides that bind with high affinity to their ligands by recognizing a specific three-dimensional structure.[156-158] Since crossing the blood-brain barrier (BBB) poses a significant obstacle to delivering therapeutic molecules to the brain, a bifunctional aptamer has been developed that targets both the transferrin receptor in the BBB (for transcytosis) and the cancer cell surface receptor epithelial cell adhesion molecule, conferring specificity to the target cell. In vivo studies in mice showed successful penetration of the bifunctional aptamers into the brain.[159]

Monaco et al. have taken this idea one step further and developed PNPs with surface aptamers. A conjugated aptamer that specifically recognizes platelet-derived growth factor receptor β (PDGFRβ) on GBM cells was manufactured to act as a nanovector for the delivery of the chemotherapeutic drug dactolisib. In vivo studies were successful in inducing specific toxicity in U87MG GBM cells in mice, and these PNPs effectively cross the BBB to arrive at the target microenvironment.[160]

Gold nanoparticles (AuNPs) have been reported that were designed with surface peptides that target both epidermal growth factor and transferrin receptors on glioblastomas. These particles, which were loaded with a hydrophobic photo-sensitizer drug, showed superior specificity and intratumoral accumulation in glioblastoma cells as compared to untargeted and monotargeted AuNPs. In vivo and in vitro work showed increased selectivity and cytotoxicity in target cells, as they also cross BBB more effectively (Figure 5a,b).[10]

Nanodiamond drug delivery platforms have also been evaluated for intracranial tumor treatment. Xi et al. described a system which consisted of doxorubicin, a chemotherapeutic agent not usually considered for treatment of brain malignancies due to its poor BBB penetration, reversibly bound to nanodiamonds for sustained functional drug release, while resulting in reduced myelosuppresion.[161] The nanodiamond-doxorubicin complexes were used with convection enhanced delivery, which is a well-described method to open the BBB transiently. Their results indicate that this system has efficacious tumor killing capacity in a bioluminescent rodent glioma model, especially when combined with convection enhanced delivery, showing improved drug distribution and retention in brain tissue compared to controls.

Theranostic strategies where nanoparticles are configured to provide diagnostic information and to deliver therapeutic nanoparticles have played important roles. Magnetic nanoparticles (MNPs) composed of ferromagnetic iron oxide (Fe_3O_4) can also be surface functionalized

with peptides or antibodies to target particular cancers and guided to a particular site using a magnetic field. If encapsulated with a drug, the MNPs can then release the agent at the desired site. This technique has been employed in in vivo models, both for therapeutic purposes as well as for MRI contrast enhancement, although BBB penetration still remains a challenge. Intracranial implantation of MNPs have also been used for targeted thermotherapy, in which temperature elevation (40–45 °C range) generated by an alternating magnetic field is used to cause cancer cell death.[12-14]

An interleukin (IL)-13 amino-coated gadolinium metallofullerene nanoparticle has been fabricated as an alternative to the commonly used gadolinium (Gd) containing materials used for MRI imaging. These nanoassemblies contain positively charged amino groups on their surfaces, which enable more efficient binding to the negatively charged phospholipid bilayers of cell surfaces compared to conventional negatively charged Gd contrast agents. This functionalized trimetallic nitride template endohedral metallofullerene (TNT EMF) was also conjugated with IL-13 (designated IL-13-Gd_3 N@ $C_{80}(OH)_x(NH_2)_y$) peptide for specific targeting of GBM in a mouse model when delivered intravenously (Figure 5c).[162] Another group has developed Gd-functionalized nanographene oxide (NGO) nanoparticles, composed of poly(amidoamine) dendrimer-grafted gadolinium-functionalized nanographene oxide (Gd-NGO), that act as effective carriers for delivery of chemotherapeutic drugs and microRNAs to cancer cells also in a GBM mouse model. The particles could also be used as a contrast agent for MRI to explore BBB opening and the extent of drug delivery to target tissues.[163]

CONCLUSIONS AND PROSPECTS

Brain tumors continue to be a common oncologic diagnosis among the pediatric population, and remain the most common cause of childhood cancer-related mortality. While multimodal therapy with chemotherapy based on tumor classification has been the standard of care, the significant intratumoral heterogeneity and possibly debilitating late effects demand a more individualized approach. New molecularly based tumor classification for some the most common childhood brain tumors and newly discovered molecular targets have resulted in multiple clinical trials using personalized or targeted approaches. Rapid advances in genetic sequencing, imaging and therapy delivery, such as small molecules targeting specific aberrant pathways, microfluidic devices for single-cell processing, targeted radiotracers, and nanodiamond systems now offer unprecedented precision in tailoring therapy for each patient. The gap between new technologies and pediatric patients consequently looms especially large, and innovative means of bridging this gap are direly needed.[164] Nevertheless, the striking need for individualized treatment has been demonstrably recognized in the raft of new personalized medicine institutes that have opened in recent years. Nowhere is this more important than in pediatric oncology, to enable every opportunity for leading basic scientists and clinicians to interact and to drive multidisciplinary efforts targeting the eradicatation of the scourge of cancer and the potentially devastating effects of its treatment.

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ABBREVIATIONS

Akt	protein kinase B
aCGH	array comparative genomic hybridization
ASL	arterial spin labeling
AT/*RT*	atypical teratoid rhabdoid tumor
AuNP	gold nanoparticle
BBB	blood-brain barrier
CAR	chimeric antigen receptor
CIMP	CpG island methylator phenotype
CLIA	Clinical Laboratory Improvement Amendment
CNS	central nervous system
COG	Children's Oncology Group
DC	dendritic cell
DIPG	diffuse intrinsic pontine glioma
DNA	DNA
DSC	dynamic susceptibility-weighted contrast-enhanced
DTI	diffusion tensor imaging
EGFR	epidermal growth factor receptor
FISH	fluorescence in situ hybridization
GBM	glioblastoma multiforme
Gd	gadolinium
HGG	high-grade glioma
IL	interleukin

IHC	immunohistochemistry
LGG	low-grade glioma
MNP	magnetic nanoparticle
MRI	magnetic resonance imaging
mRNA	mRNA
MS	methylation-specific
mTOR	mammalian target of rapamycin
MPLA	multiplex ligation-dependent probe amplification
NCI	National Cancer Institute
NGO	nanographene oxide
NTRK2	neurotrophic tyrosine kinase type 2
PCR	polymerase chain reaction
PD-1	programed cell death protein-1
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
PDGFR	platelet-derived growth factor receptor
PET	positron emission tomography
PNP	polymeric nanoparticle
RNA	ribonucleic acid
SHH	sonic hedgehog
UMI	unique molecular identifier
WHO	World Health Organization

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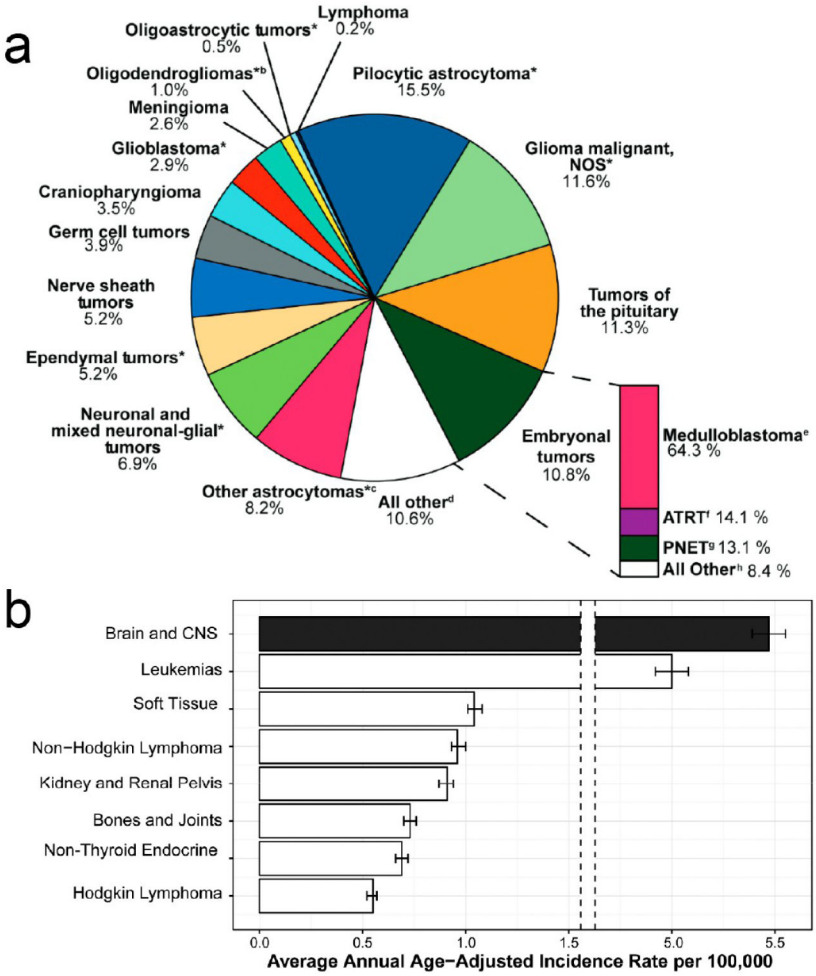

Figure 1.

(a) Incidence of pediatric (age 0–19) central nervous system (CNS) tumors by histological subtype. Of the three main categories, gliomas are the most common (53.1% of diagnoses), followed by embryonal tumors (13.8%) ependymal tumors (5.8%). (b) Average annual age-adjusted mortality rate of all primary brain and CNS tumors in comparison to other common cancers for children age 0–14 years. Reprinted from ref 20 by permission of Oxford University Press, Copyright 2016.

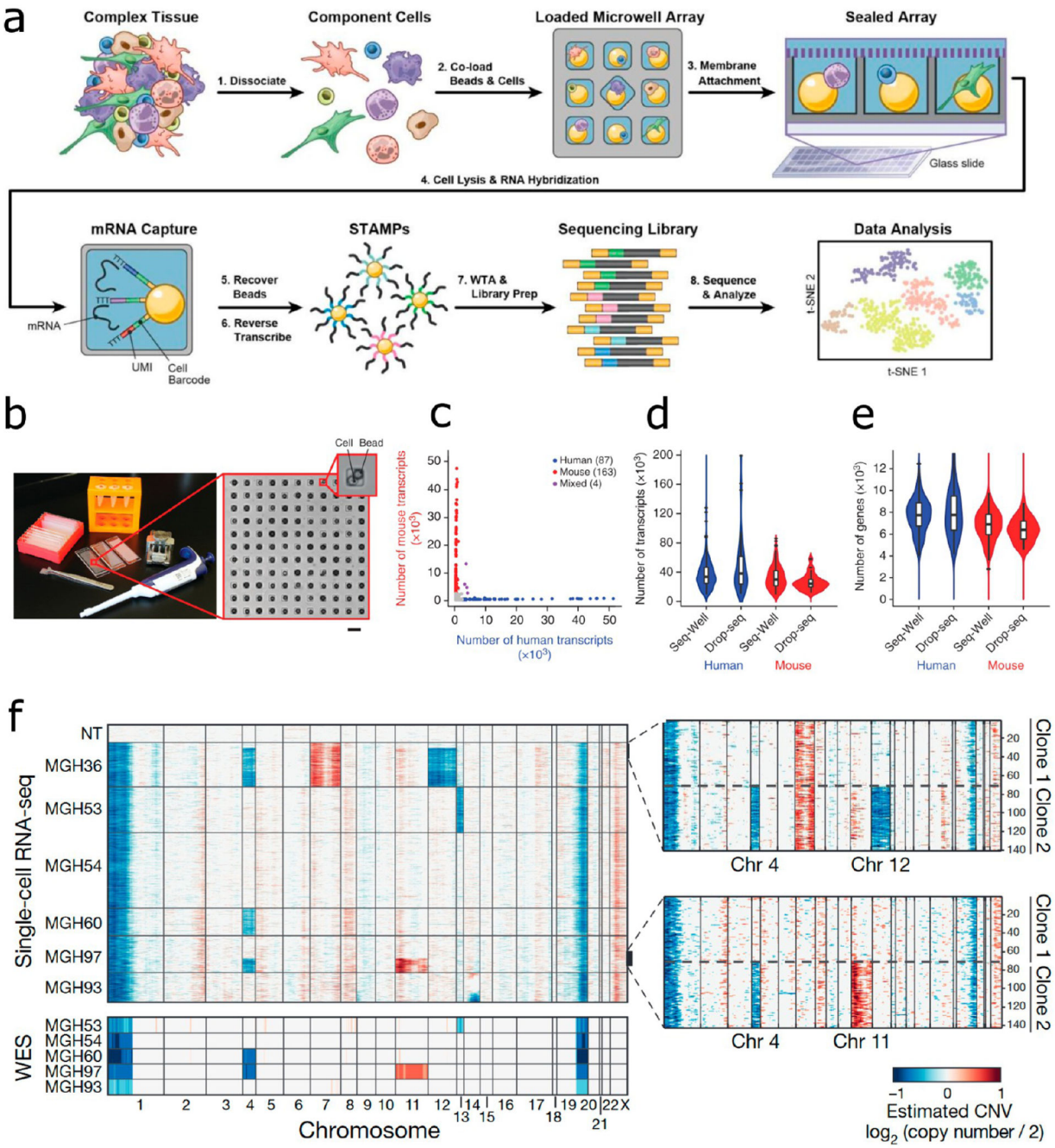

Figure 2.

(a) In Seq-Well, cells are obtained from complex tissues or clinical biopsies, and digested to form a single-cell suspension. Barcoded mRNA capture beads are added to the surface of a microwell device, settling into wells by gravity, and then a single-cell suspension is applied. The device is sealed using a semipermeable membrane that confines cellular mRNAs within wells while allowing efficient buffer exchange. Liberated cellular transcripts hybridize to the bead-bound barcoded poly deoxythymine (dT) primers that contain a cell barcode and a unique molecular identifier (UMI) for each transcript molecule. After hybridization, the beads are removed from the array and bulk reverse transcription is performed to generate

single-cell cDNAs attached to beads. Libraries are then made by a combination of polymerase chain reaction (PCR) and tagmentation, and then are sequenced. Afterward, single-cell transcriptomes are assembled *in silico* using the cell barcodes and UMIs. (b) Equipment and arrays used to capture and lyse cells, respectively, in Seq-Well. Scale bar = 100 μm. (c) Sequencing mix of human and mouse cells demonstrates distinct transcript mapping and single-cell resolution. (d) Number of trancsripts and (e) genes detected in single-cell libraries generated by Seq-Well or Drop-seq. (f) Representative single-cell RNA-seq of cancer and noncancer cells in six oligodendrogliomas. On the left, copy number variant profiles inferred from single-cell RNA-seq and DNA whole-exome sequencing of the six oligodendrogliomas. On the right, analysis of copy number variants identified two subclones of cells in tumors identified as MGH36 and MGH97. Panels (a)–(e) reprinted from ref 62 by permission from Macmillan Pubishers Ltd.: *Nature Methods*, Copyright 2017. Panel (f) reprinted from ref 165 by permission from Macmillan Publishers Ltd.: *Nature*, Copyright 2016.

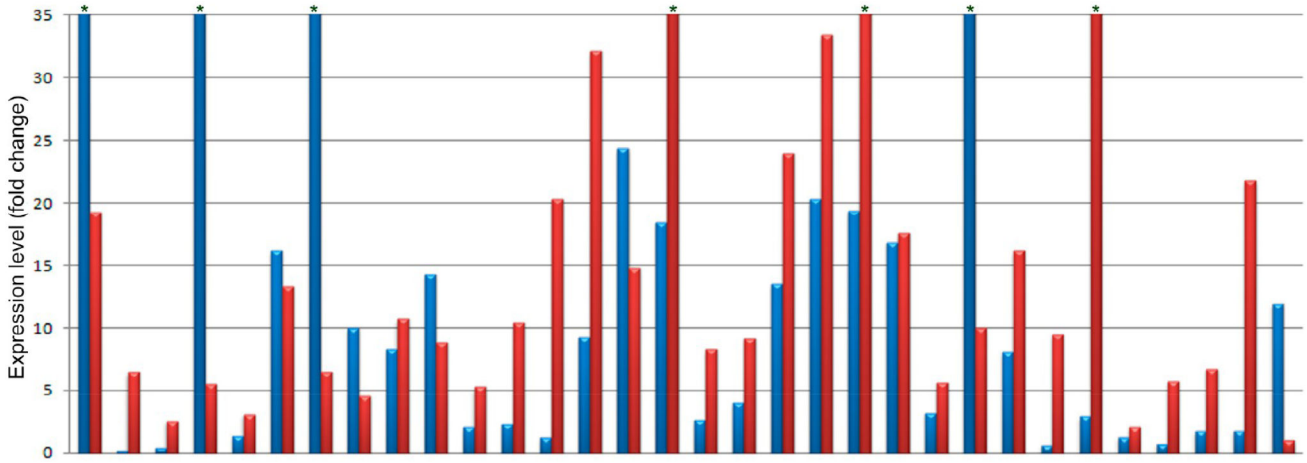

Figure 3.

Tumor and serum microRNA-720 expression in individual glioblastoma multiforme (GBM) patients. PCR-based microRNA microarrays and real-time qPCR were performed in triplicate on complementary DNA amplicons created from RNA extracted from each GBM tumor specimen and intraoperative serum sample using a human serum albumin-microRNA-720 probe. Blue bars indicate mean tumor microRNA-720 fold-change expression, red bars indicate mean serum microRNA-720 fold-change expression. Asterisk (*) denotes >35-fold higher expression than the normative standard. Figure courtesy of A. C. Wang, preliminary data.

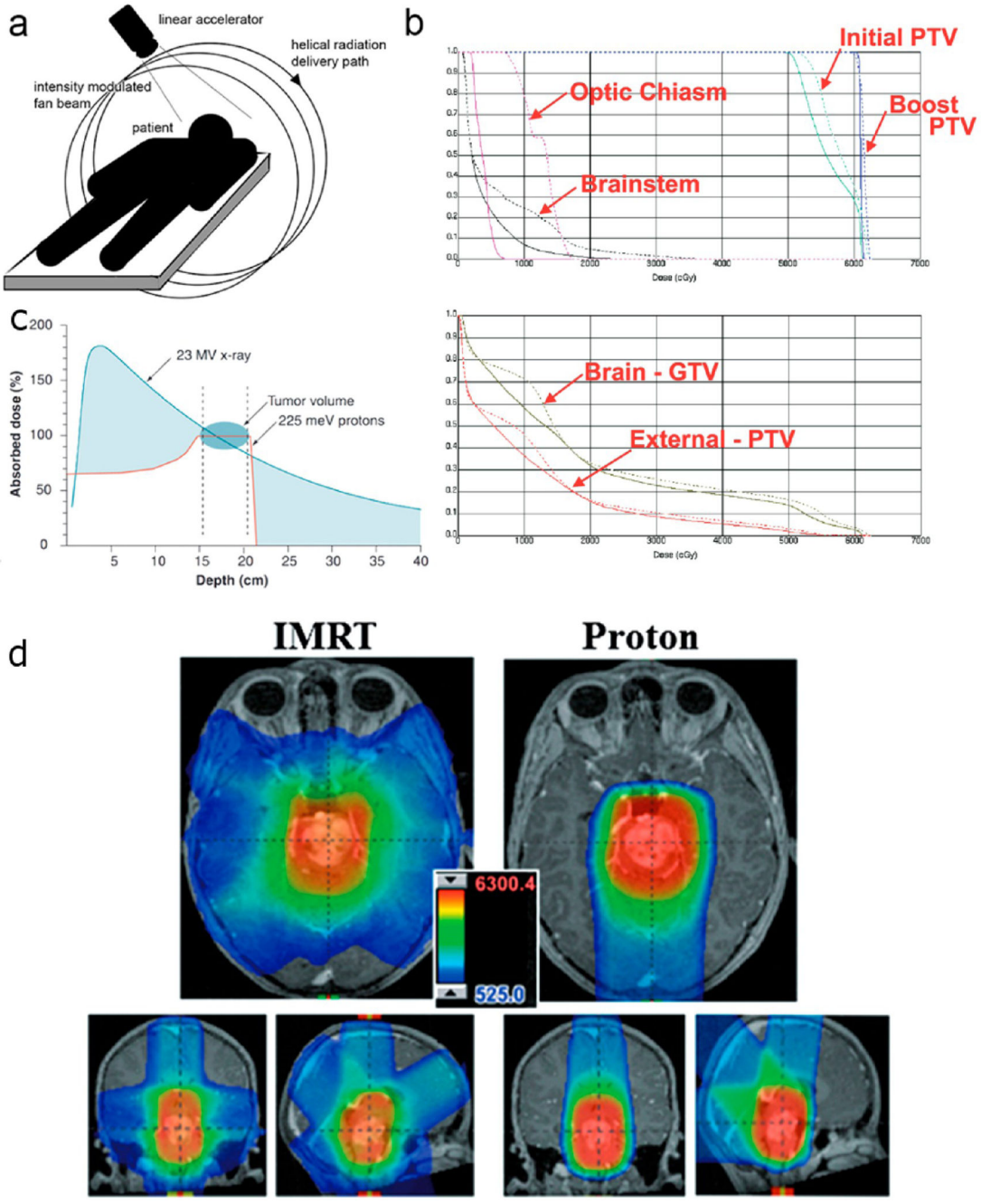

Figure 4.

(a) Illustration of helical tomotherapy as an intensity-modulated radiation therapy device where a linear accelerator continuously revolves around the patient, while slowly advancing the patient through the plane of rotation. For radiation therapy dose delivery, a collimator is used to allow only sections of the fan beam to reach the patient. The collimator pattern changes as a function of gantry position, which provides many degrees of freedom to deliver highly conformal dose distributions. (b) Representative dose volume histograms comparing conventional three-dimensional conformal radiotherapy (dashed line) versus intensity-modulated radiotherapy (solid line) plans for a patient with a left parietal lobe tumor. Note

the dose reduction for uninvolved parts of the brain. (c) Comparison of dose distribution using a proton beam. Note that ionization increases as the proton beam enters the patient, reaches intended dose at the tumor, then declines as velocity decreases. (d) Comparison of a photon- and a proton-based radiation therapy plan for a pediatric patient with a supratentorial ependymoma. Representative axial, coronal, and sagittal slices are shown for each plan. Approximate percentage isodoses are shown for reference in the axial slices. PTV: planning target volume. Brain, GTV: total brain without gross tumor volume. External, PTV: total tissue volume without planning target volume. IMRT: intensity-modulated radiotherapy. Panel (b) is reprinted from ref 81 with permission from Elsevier, Copyright 2007. Panel (c) is reprinted from ref 83 with permission from Taylor & Francis Ltd., http://www.tandfonline.com, Copyright 2010. Panel (d) is reprinted from ref 80 by permission from Taylor & Francis Ltd., http://www.tandfonline.com on behalf of Acta Oncologica Foundation, Copyright 2013.

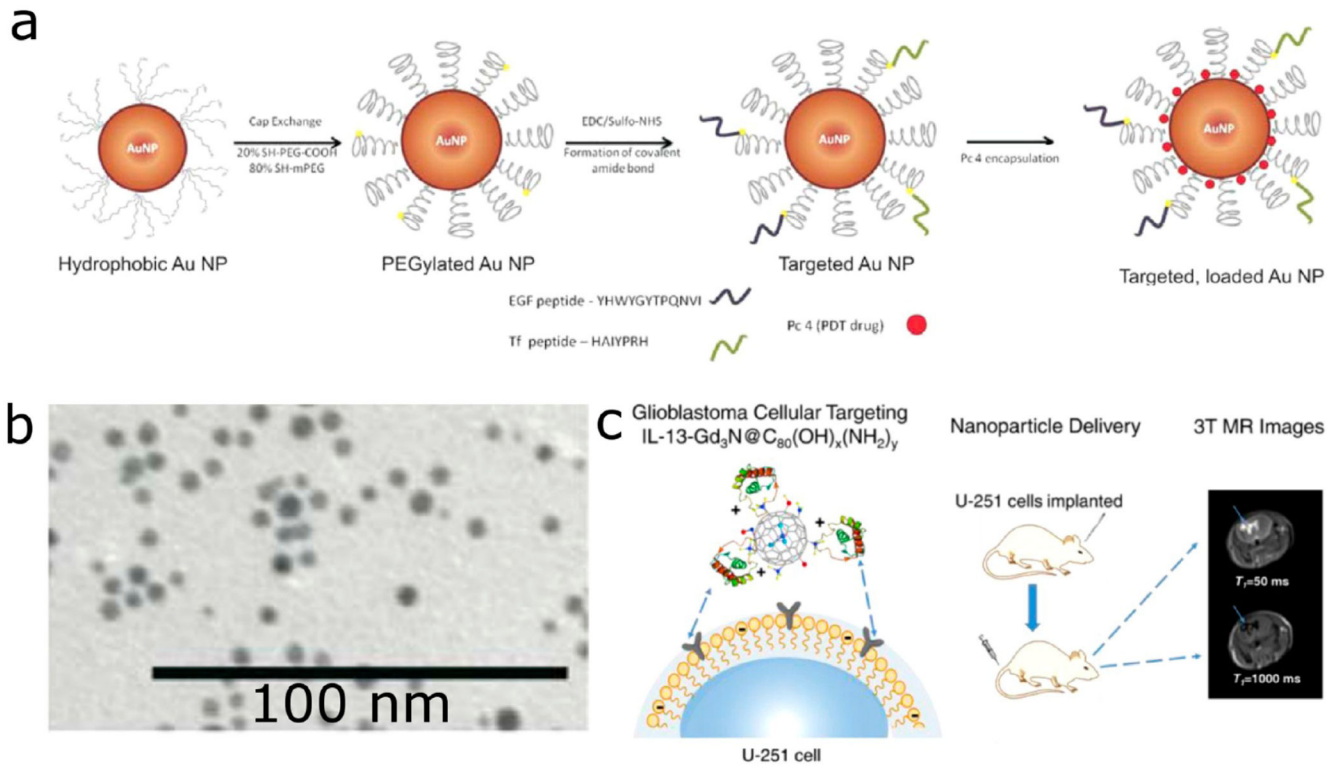

Figure 5.

(a) Schematic illustrating the design of a dual-targeted gold nanoparticle (AuNP) system used to target glioblastoma (GBM) cells. The particles are functionalized with multiple receptor binding peptides to address intratumoral heterogeneity of GBM populations and the photosentizer phthalocyanine 4. (b) Transmission electron microscope image of hydrophobic gold nanoparticles used to prepare dual-targeted AuNPs. Scale bar = 100 nm. (c) Schematic illustrating nanoparticle targeting of U-251 glioblastoma cells. Localization of the gadolinium-tagged nanoparticles to glioblastoma cells implanted into mouse models is monitored via magnetic resonance imaging. Panels (a) and (b) reprinted with permission from ref 10. Copyright 2015 American Chemical Society. Panel (c) reprinted with permission from ref 162. Copyright 2015 American Chemical Society.

Table 1.

Open Clinical Trials Utilizing Targeted Molecular Therapies for Pediatric Brain Tumor Patients[a]

drug name	molecular target	tumor entity	ClinicalTrials.gov identifier	trial phase
vismodegib	smoothened receptor	medulloblastoma		Phase II
TB-403	placental growth factor	medulloblastoma, others		Phase I, II
tazemetostat	histone-lysine methyltransferase EZH2	rhabdoid tumors, including AT/RT		Phase I
alisertib	aurora A kinase	AT/RT		Phase II
K27M peptide vaccine	histone 3.3K27M epitope	diffuse midline glioma, other gliomas		Phase I
panobinostat	histone deacetylase	diffuse midline glioma		Phase I
vemurafenib	BRAFV600E	glioma		Early phase I
selumetinib	MEK1	low-grade glioma		Phase II
dabrafenib + tremetinib	BRAF + MEK	high-grade glioma		Phase II

[a]Note that this table is not comprehensive and only lists therapies discussed in this Review.