Chapter Six: General Discussion

In this Chapter, I summarise the main findings from this Thesis, discuss potential future directions of transposon mutagenesis screening particularly in the context of gliomas, and also the challenges faced in developing treatments for these tumors.

In this thesis, I have focused on the role of *EGFR*, particularly its activating mutation *EGFRvIII*, in gliomagenesis and how it requires cooperative genetic partners for cancer progression. Note the cell of origin for gliomas was not the focus of my work and so will not be discussed further in this Chapter. The main advance of this body of work is showing that *EGFRvIII* can act as an initiating event in brain tumorigenesis without the prior introduction of tumor suppressor losses. There is a relatively long latency for development of fully-formed gliomas and a low incidence of high-grade tumors, implying a requirement for subsequent additional driving genetic events. Possible reasons why this was not observed in previous studies are discussed in Chapter 3, but include the longer observation times in our study, and the use of the *nestin*-cre driver to for early expression of *EGFRvIII*. We also demonstrated for the first time that *EGFRvIII* can cause spinal glioma formation. Further work should include investigating the cell of origin for these tumors in this mouse model, as previously discussed. Through whole-exome sequencing, we identified these tumors somatically acquired recurrent mutations in *Trp53*, *Tead2* and *Sub1* (all of which have recurrent alterations in human gliomas), as well as deletions in *Cdkn2a* and *Nf1*, and amplification of *EGFRvIII*. RNA-sequencing of these tumors showed aberrant expression of homeobox genes and enrichment of pathways for regulating cell differentiation, as well as known oncogenic pathways including MAPK, p53 signalling and Jnk pathways. We next performed an *in vivo* genome-wide forward genetic screen for *EGFRvIII*-cooperative drivers using a conditional *piggyBac* transposon insertional mutagenesis system. Sequencing and analysis of the *piggyBac* integration sites in 96 gliomas identified a panel of 281 genes which were common integration sites (CIS).

Analysis of the CIS in the PB-cohort provided strong evidence of a number of known and unknown putative genetic drivers collaborating with *EGFRvIII*. Although functional validation of individual genes are needed to definitively support them as drivers, there are multiple lines of evidence which support our conclusion. First the observation of integration sites in the same genes in a significant fraction of tumors provides strong statistical support for selection of these mutations in gliomagenesis. Second, the position of these integrations with respect to the gene body and consequence on expression, consistently disrupting or activating gene expression, such as disruption of *Nf1* and another Ras-inhibitor *Spred1*. Third, data from RNA-seq support the integration pattern because the transposon is designed to promote the expression of the gene, such as those seen with transcripts emanating from the transposon which splice into *Rad51b* or cases where transcripts from the gene splice into the acceptor sites encoded by the transposon thereby disrupting gene expression such as *Cdkn2a*, *Nf1*, *Pten*, *Sox6*, *Sox5*, *Spred1*, *Qki* and *Ust*. Fourth, the overlap of genes identified with mutations / focal deletions by exome sequencing and mutated by *piggyBac* cross-validates their biological selection – including *Cdkn2a*, *Cacul1*, *Esr1*, and *Myo10* (focal deletions); *Nf1*, *Prex2* and *Dgkb* (recurrent mutations); *Cdkn2a*, *Nbn*, *Enc1* and *Spag17* (single mutations). Finally, the correlation with human genetic data is compelling, not only for the previously described genes but also for genes like *SPRED1*, *TCF12* and *SOX6* which are deleted in 27%, 23% and 18% of GBMs, respectively. Interestingly, *piggyBac* identified multiple tumor suppressors co-deleted in large regions in human tumors including *SPRED1* and *TCF12*, and *QKI* and *UST*. The conserved role of these genes in both species validates the similarity and therefore relevance of the mouse model to the human disease.

Comparison of CIS between brain and spinal gliomas revealed that these two types of tumor share many common core drivers such as *Cdkn2a* and *Nf1*, but otherwise each have a some unique putative driver genes acquired later in tumor evolution (although these require further functional validation). We validated *Pten* as a novel cooperative driving event with *EGFRvIII* in spinal tumors, whereas previously this role for *Pten* was only proposed for brain gliomas. The

putative driver genetic events in this work will also provide a comprehensive gene list for further mechanistic work into how genetic alterations support glioma progression.

In order to provide conclusive evidence of these novel genes as drivers in glioma, it would be worthwhile generating conditional knock-out mice in genes of the most convincing CIS. These mice could then be crossed with *EGFRvIII* and *nestin*-cre to demonstrate whether tumorigenesis is accelerated as would be expected; we showed this for *Pten* which accelerated spinal gliomagenesis in particular with *EGFRvIII*. More mechanistic studies could also be done using these models, for example RNA-sequencing analysis may demonstrate different or additional pathways are activated in these tumors compared with those initiated with *EGFRvIII* alone. Producing such mice may be suitable only for relatively small numbers of candidate genes, as it is expensive and time-consuming to produce larger numbers of conditional knock-out mice. An alternative method for potentially validating more candidate genes more efficiently is to use somatic genome engineering with CRISPR-cas9. This method has been applied to producing glioblastomas *in vivo* in mice through targeting known drivers (*Trp53*, *Pten* and *Nf1*) for knock-out as a proof-of-principle; the same study also generated medulloblastomas in mice through CRISPR-mediated somatic disruption of *Ptch1* [264]. Even more recently, Chen and colleagues have used a pooled CRISPR library to screen for driver genes of GBM by stereotaxic injections into the brain of mice; the pool contained sgRNAs for pan-cancer tumor-suppressor genes from TCGA but excluded oncogenes given that this method is for gene disruption rather than activation [265]. The results were able to profile which of the pan-cancer genes are most relevant for GBM. It is conceivable that we could apply this method of CRISPR pooled libraries to validating our list of 281 glioma CIS genes, either as a complete set or for subsets of these genes; given our list also has known and putative oncogenes such as *Pdgfra*, it may be worth considering a separate oncogene screen using activating versions of cas9 that have been shown to have efficacy in conducting functional screens [83, 266].

Spontaneous Mutations versus Transposons in Cancer Gene Discovery

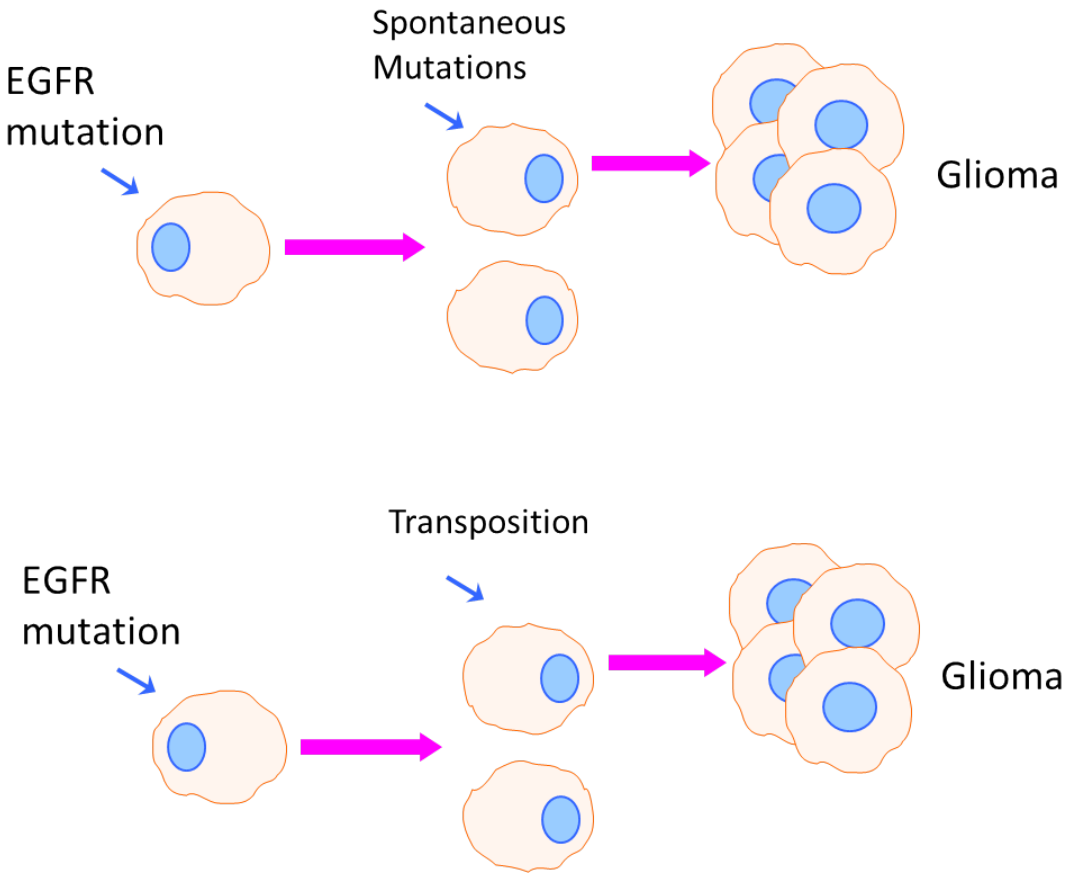

Figure 1. Comparing methods of tumor evolution in model organisms. After acquiring an *EGFR* mutation, tumor precursors will acquire genetic alterations that can be selected for through Darwinian natural selection principles; alternatively, transposition can accelerate these alterations. Tumors were sequenced to identify the genes driving cancer in both cases.

In this PhD, after discovering *EGFRvIII* was sufficient to initiate gliomagenesis in mice, I also used whole-exome sequencing to determine the additional genetic alterations that are acquired during tumor evolution whilst in parallel conducting a forward genetic screen with transposon mutagenesis to determine cooperative genetic drivers with *EGFRvIII*. We demonstrated that in the presence of transposon mutagenesis, there were significantly fewer spontaneous genetic alterations in resulting tumors, likely because transposon insertions were being selected for in

tumorigenesis over spontaneous alterations. These common integration sites revealed the known glioma genes in addition to novel candidate drivers; nevertheless, spontaneous genetic alterations revealed by whole-exome sequencing also occurred in cancer genes. Amongst the top mutated genes in these tumors was *Trp53*, which occurred less frequently in tumors with transposition. This difference in spectrum of mutations between the two tumor cohorts is likely to reflect the increased selection for transposon insertions in cancer genes in the EGFR-PB cohort. Moreover, the other top mutated genes in *EGFRvIII*-only tumors, such as *Tead2* and *Nt5c2* (also frequently aberrated in human gliomas) were not common integration sites from transposition. These findings highlight that whole-exome sequencing from tumors in mice and transposition-induction of tumors are potentially complementary methods of cancer gene discovery, which together are powerful tools for cancer gene discovery.

Although it still remains a major challenge to infer cooperation cancer genes from human genomic studies alone, such as between *EGFR* and other drivers, there are alternative approaches to answer this question compared with our approach here. A recent elegant study by Blakely and colleagues analysed genomes from 1,122 *EGFR*-mutant lung cancers from human patients and found that in addition to *EGFR*, the majority of tumors carried co-occurring genetic alterations in other driver genes such as *CTNNB1*, *PIK3CA*, *RAF*, *MET* and *MYC* [267]. These findings led the authors to conclude that such lung cancers are not single-driver gene entities, but rather have co-occurring driver events. The strength of this work comes from analysing a large number of patient tumors in order to determine significant co-occurring alterations. If a similar study were to be performed for *EGFR*-mutant gliomas, it would potentially provide a strong way of cross-validating our results from this study in patients. However, it must be borne in mind that there are challenges with interpreting human cancer genomes, in particular identifying driver genes from passenger genes, and identifying rare cancer genes amongst large genomic amplifications or deletions. Therefore, studies in mice provide complementary tools for identifying driver genes.

Novel developments in transposon mutagenesis screening

There are a number of recent developments in transposon mutagenesis screening that advance its utility for cancer gene discovery. An elegant study employed a single-copy of the *Sleeping Beauty* transposon per cell as part of a whole-body mouse cancer screen for genes cooperating with *Pten* in driving prostate, skin and breast cancers [67]. This model had several advantages, not least that having only one copy of the transposon per cell reduced the number of passenger mutations, helping prioritisation of the strongest candidate cancer genes for further functional validation. Another advantage was that the transposon was coupled to *Pten* inactivation in the same genome, which may increase the sensitivity of the screen for identifying *Pten*-cooperative cancer genes. Moreover, the transposon lacked a strong promoter for driving endogenous gene expression, and thus was an inactivating-only transposon. Although this meant the screen was not designed for finding putative oncogenes, it greatly simplified downstream analysis and interpretation of common integration sites, which all reflected putative tumor suppressor genes. Further exploration of this model is warranted to confirm reduced passenger mutations induced by the transposon and to compare the sensitivity of this screen for discovering cancer genes compared with models with multiple transposon copies (such as the screen I have presented in this Thesis).

Another recent advance, of particular importance for the brain cancer field, was the use of transposon mutagenesis screening for identifying drivers of medulloblastoma at recurrence after treatment [268]. The investigators used a *Sleeping Beauty* model to produce medulloblastomas in mice; they microsurgically removed these tumors and treated the mice with radiotherapy, reflecting the standard of care in human patients with this disease. As expected, medulloblastomas recurred after this treatment. Genetic sequencing revealed different common integration sites between primary and relapsed medulloblastomas. In keeping with this, genomic sequencing of human primary and relapsed medulloblastomas revealed different mutations. These data suggests distinct genetic drivers are inducing a primary as opposed to a relapsed medulloblastoma. Moreover, they found that the dominant clone of relapsed medulloblastomas arose partly through clonal selection (imposed presumably by surgery and radiotherapy) of a

minor subclone present in the primary tumor. Implications of these findings are that treatments aimed at truncal mutations in the primary tumor are unlikely to provide cures if they are not present in the relapsed tumors, advocating the need for repeated tumor biopsy at tumor recurrence. Future studies using transposon mutagenesis to identify the molecular players driving tumor recurrence in other contexts, such as different treatments and different cancers, are warranted.

Given that *piggyBac*, like other transposons, continues to mobilize around the genome in the presence of transposase, it is also useful as a system for determining resistance mechanisms to chemotherapeutic agents. A recent study demonstrated this for *Trp53-Mdm2* resistance mechanisms in an $Arf^{-/-}$ model, in which *piggyBac* common insertions were found in *Trp53* and *Bcl-xl*, the latter of which were activating insertions [269].

Despite the wealth of useful data provided *in vivo* transposon-based cancer screens in mice, these studies are typically expensive, time-consuming and resource-heavy, given that multiple mouse crosses are required demanding relatively productions of relatively large numbers of mice. For these reasons, there is increased demand for reliable, *in vitro* transposon mutagenesis models for performing cancer screens. Useful advances on this front have been made recently. For example, Fan et al have reported a *piggyBac* screen with an *EGFR*-mutant lung cancer cell line in the presence of an EGFR inhibitor; sequencing and analysis of the transposon integration sites in this cell line identified *MET* activation (known to drive resistance to EGFR inhibitors) as well as a novel player, *YES1* (a Src family kinase) [270]. The investigators then processed human clinical datasets of lung cancer patients treated with EGFR inhibitors, and identified the presence of *YES1* amplification in a subset of these patients. Treatment of an EGFR-mutant lung cancer cell line containing activating *YES1* insertions with a YES1 inhibitor or *YES1* siRNA knockdown sensitised the cells to treatment with EGFR inhibitors, supporting the role of YES1 in driving drug resistance. This screen is a clear demonstration of the utility of *in vitro* transposon screens for identifying genetic drivers of treatment resistance. There are several such reports of *in vitro* transposon-based cancer screens [271-274], supporting the promise of these tools for cancer screening.

EGFR inhibitors have proved to be successful in some cancers that carry *EGFR* mutations, such as lung and colorectal cancers, but have not shown to improve survival in GBM. In the latter case, there must be mechanisms for tumor cells to resist growth inhibition by these drugs, although these mechanisms are poorly understood. In this PhD, I established multiple primary cultures from *EGFRvIII*-PB tumors; these can be expanded *in vitro* and then injected into mice that can be treated with EGFR inhibitors (or the cells can be directly treated with these drugs for *in vitro* screening). As tumors develop *in vivo* in the presence of continued drug treatment, they will develop genetic alterations driven by *piggyBac* insertions that will enable them to escape growth inhibition by EGFR inhibitors. Sequencing for *piggyBac* common integration sites in the resulting tumors will help identify these genetic drivers of drug resistance. Understanding these genetic alterations may help enable design of rational combinatorial therapies involving EGFR inhibitors for treating GBM patients.

Another important aspect of our work worth exploring in future is how the nature of the initiating driver mutation in gliomas affects the timing and nature of subsequent genetic drivers. It is clear that many driver genes are only acting as such in particular contexts, such as in cooperation with other genes like *EGFR*. Therefore, depending what the initiating cancer mutation is, a cancer is likely to be predisposed to evolving in a particular way with clonal selection for certain mutations over others. This hypothesis is challenging to explore in end-stage tumors from patients using statistical methods with sequencing data alone. Rather, modelling in mice carrying these sensitizing mutations is an orthogonal and potentially clear-cut way for tackling this challenge. Given the time-constraints of completing a PhD and the long period of time needed for crossing mice and generating tumors, it was not possible to complete the *Trp53*-transposon screen for this thesis. This project will hence be on-going and once the results of this genome-wide forward genetic screen are known, it would be interesting to compare the CIS from *Trp53*-induced gliomas with those of *EGFRvIII* gliomas. A *Sleeping Beauty* screen for intestinal cancer drivers discovered that there were different patterns of CIS genes depending on which sensitizing mutations were

carried by the mice (*Apc*, *Smad4*, *Trp53* or *Kras* mutations), consistent with the notion that the founding mutation influences the genetic evolution of a tumor [53].

EGFR as a therapeutic target in gliomas

Given that *EGFR* was the first oncogene to be associated with glioblastoma (GBM), it is worth considering the therapies directed against EGFR that have been and are being developed for treatment of this disease. In the period when the first oncogenes in cancer were being described, it was discovered that the protein sequence of EGFR was similar to the viral oncogene, v-erb B, suggesting EGFR itself may have oncogenic activity[91]. Since then amplifications and various mutations, particularly truncating mutations that cause constitutive activation of the receptor, were described in up to 60% of GBMs. The *EGFRvIII* variant attracted particular interest, given that deletion of exons 2 -7 in this mutant leads to a novel antigenic epitope that is specific to this cancer and not expressed in normal tissues, forming a GBM 'signature molecule'. Various methods of targeting *EGFR* amplification and / or *EGFRvIII* have been developed, including small-molecule tyrosine kinase inhibitors, monoclonal antibodies, conjugated antibodies, CAR-T cells and vaccines. I will discuss the key agents, the challenges faced with these therapies, and potential future directions for EGFR-based therapies in glioblastoma.

EGFR as small molecule inhibitor target

A number of small molecule tyrosine kinase inhibitors (TKIs) are available and approved for a variety of cancers, including colon, pancreas and lung, although none are thus far approved for the treatment of glioblastoma due to disappointing results in clinical trials to date. The main such agents include erlotinib, gefitinib, afatinib and lapatinib. Erlotinib alone demonstrated no clinical efficacy in a trial in newly diagnosed GBM patients [143], and gefitinib did not improve overall survival in a phase II trial[275]. Afatinib and lapatinib showed very limited efficacy as single agents in early clinical trials in recurrent GBMs [276, 277].

A major problem with these TKIs for treating GBMs is poor brain (and more specifically tumor) penetrance in human patients. This itself is also difficult to measure, save for novel mass spectrophotometric methods that can be applied in animal models to more accurately measure drug-tissue concentrations. In clinical trials including tissue measurements of erlotinib and gefitinib, available from recurrent tumor resections, the concentration of erlotinib in GBM was only 5-7% that of the plasma, which may at least partly explain its poor results, although the concentration of gefitinib in GBM tissue was better (2 – 3 times the plasma concentration)[278]. Another important challenge is that the fact that these cancers display an 'adaptive' capacity: GBM cells activate many redundant pathways (and also genes in the same pathway, such as *Nf1* and *Spred1* in the Ras pathway as we found in our mouse gliomas), so they can overcome inhibition of a single molecule within one of these pathways.

EGFR as an immunotherapy target

Monoclonal antibodies can be developed in different ways to produce different effects on their target, such as blocking a receptor (in this case EGFR) and preventing ligand binding, causing internalisation and degradation of the receptor, binding the target and activating antibody-dependent cell-mediated cytotoxicity (ADCC), or binding the target and causing cell damage through a conjugated toxin.

Cetuximab is a monoclonal antibody used in colorectal cancer and has been trialled in GBM; it is a blocking antibody for EGFR. In orthotopic xenograft models of GBM, cetuximab in combination with VEGF inhibition led to reduced tumor migration and invasion[279]. However, in clinical trials cetuximab did not improve outcomes in recurrent GBM either as a single agent or with other agents [280, 281]. A recognized difficulty in using cetuximab for GBM is also related to tumor penetrance in the central nervous system (including overcoming the blood-brain barrier). Therefore, current developments underway are aimed at improving tumor tissue concentrations of cetuximab, including direct intracranial infusion of the antibody, intracranial injection of adenovirus containing the antibody gene so that transduced cells produce the antibody to

increase local concentrations, and selective osmotic blood-brain barrier opening with intra-arterial mannitol infusion and then cetuximab infusion. It remains to be determined whether these methods will improve clinical efficacy of the antibody.

Nimotuzumab is another EGFR blocking antibody, which differs from cetuximab in having a lower binding affinity for EGFR and is therefore more selective for targeting EGFR-overexpressing cells (as in GBM) compared with normal cells that also express EGFR[282]. It showed potential efficacy in a phase II trial and also in a randomised phase III trials using nimotuzumab in addition to standard therapy (radiotherapy and temozolomide) for GBM[283, 284]. It is currently being explored further in subgroups of patients, including paediatric diffuse intrinsic pontine glioma.

A promising avenue for therapeutic based on EGFR as a target in GBM is the engineering of T-cells to express a chimeric antigen receptor to recognise a target independently of MHC-mediated antigen presentation, named chimeric antigen receptor T-cells (CAR-T-cells). This has shown efficacy in certain cancers such as leukaemia[285]. EGFRvIII in GBM is a particularly attractive target for this approach given that it is a unique antigen that is specific for the cancer and not expressed on normal tissues. CAR-T-cells against EGFRvIII are in development and some are in early phase clinical studies[286].

As mentioned, EGFRvIII contains a unique epitope that does not occur in normal tissues; as such, a vaccine, rindopepimut, has been developed containing the unique amino-acid sequence of EGFRvIII[145]. When this peptide is injected intradermally, it has been shown that an immunologic response against the peptide is mounted, which can lead to immune-mediated destruction of EGFRvIII-positive GBM cells[287]. Although rindopepimut showed good results in early phase trials, the recent phase III trial did not show improvements in overall patient survival with this vaccine[288].

The reasons for lack of success of these various agents targeting EGFR are complex. Aside from the issue of drug delivery into GBMs (requiring passage through the blood-brain barrier), another major challenge is the intratumoral heterogeneity in *EGFR* expression. This heterogeneity has been observed for EGFRvIII expression, in that although *EGFRvIII* is common amongst GBM

patients, it is not expressed in all tumor cells[289], so therapies such as the EGFRvIII vaccine do not lead to destruction of all GBM cells. More recent sequencing studies have identified other activating *EGFR* mutations in GBM, apart from *EGFRvIII*, including exon 12-13 deletion, exon 14-15 deletion, and C-terminal deletion of exon 25-27, as well as point mutations and gene fusions involving *EGFR* [27, 290]. These various *EGFR* mutations can co-exist in one GBM – single cell sequencing analyses have found that up to 32 different tumor subpopulations can be present in a single GBM biopsy with each one containing a different pattern of *EGFR* mutations[291]; this complexity presents potential mechanisms for resistance to single EGFR-targeting therapies. Another problem is the documented co-expression of multiple receptor tyrosine kinases in GBMs, including MET and PDGFRa in addition to EGFR[292]. Thus, combinations of small molecule inhibitors targeting multiple RTKs are likely to be more successful than single agents. Some evidence has also emerged that GBMs can develop resistance to EGFR inhibitors because these cells can carry the EGFR amplification on double minutes (extrachromosomal DNA); when these tumors are treated with EGFR inhibitors, the cells lose their double minutes containing the EGFR amplification, and when treatment is stopped these double minutes can re-appear[149].

A very recent study aimed to elucidate the pharmacogenomic landscape of patient-derived tumor cells (PDCs) from 385 tumors across 14 cancer types [293]. The study demonstrated these cells reflected the genetics and biology of the disease more accurately than cancer cell lines and patient-derived xenograft models. Subgroup analysis of *EGFR*-altered GBMs found that *EGFR* amplification, *EGFRvIII*, *EGFR* point mutations and fusions all predicted sensitivity to multiple EGFR inhibitors. Moreover, they found that in *EGFR*-altered GBM PDCs resistance to EGFR inhibitors could be overcome by the use of ibrutinib, a drug currently used in haematological malignancies that acts by inhibiting phosphorylation of Bruton's tyrosine kinase (BTK). *EGFR* amplification and *EGFRvIII* both conferred sensitivity to ibrutinib in GBM PDCs suggesting equivalent driving effects of these types of *EGFR* alteration. Given ibrutinib is able to cross the blood-brain barrier, this is a potential therapeutic approach worth exploring, although testing *in vivo* in genetically faithful models is required.

Future Challenges in Glioma Management

Over the last few years, the genetic landscape of gliomas has been the subject of intense investigation and it is very likely that the major drivers of these tumors will be defined more clearly over the next decade. The question arises then of what should we do with all of these data? Of course, it will be important to tailor what we know about these tumors as a population to individual patients, who will carry their own cluster of driver mutations in their tumor. As our knowledge and understanding of these tumors improves, so does our classification of the tumors into distinct subtypes based on the molecular data [34, 93, 173, 294]. Indeed, recent detailed molecular characterization of CNS primitive neuroectodermal tumors (PNETs) led to discovery of distinct new brain tumor identities [295]. Having highly specific classifications will enable us to make our management of patients more personalized, ideally with prognosis of patients being accurately reflected in the molecular subtype of glioma they have. Even more advanced than this would be to personalize therapy for patients based on the molecular profile of their tumor; at the most comprehensive level, this would involve whole-genomic profiling and transcriptomic sequencing of each patient's tumor, not only for the most accurate classification but also potentially to give drugs based on their unique genetic and epigenetic tumor profile. Undoubtedly, this will be complicated by issues such as rare subclonal driver mutations and intratumor heterogeneity, which is particularly marked for glioblastomas compared with other cancers. Drugs may require re-engineering in order to penetrate the blood-brain barrier. What does this mean in practice – will we require sequencing of tumors from multiple sites for every single patient in order to best select a therapy based on common genetic alterations across most sites? Taking the example of EGFR directed therapy, it has been noted that for tumors carrying the *EGFRvIII* mutation that not all cells in the tumor actually express the mutant protein and therefore giving these patients EGFR inhibitors is unlikely to lead to cure since not all cells will be inhibited by these drugs, leaving aside the complex resistance mechanisms cancers can develop after this and the difficulties with drug penetrance into the tumor. Other problems with approaches involving inhibition of oncogenes are that for oncogenes acquired early during carcinogenesis the tumors may no longer be dependent on these oncogenes for growth as they have acquired many more drivers, making early ones redundant. This would demand the need

for targeting of multiple independent cancer genes in order to have a durable suppression of tumor growth[144]. A further problem is that many of the altered genes found in gliomas, including in my work here with *piggyBac* mutagenesis and identification of mutations in mouse gliomas, are tumor suppressors rather than oncogenes. These genes are more difficult to target therapeutically, as they may require re-expression rather inhibition (which can be done using drugs in many cases). However, tumor suppressors (and their downstream pathways) are increasingly regarded as potentially powerful therapeutic targets [296], particularly if a definite structure such as a pocket can be identified, as exemplified by molecules blocking the interaction of p53 with MDM2 thus increasing wild-type p53[297]. Targeting the downstream activated pathway following loss of a tumor suppressor gene can also be an attractive approach, as exemplified by PI3K inhibitors which are being explored as therapeutic options in cancers with *PTEN* loss. Indeed, the confirmation that *Pten* loss accelerates leptomeningeal spinal LGG growth *in vivo* in my work suggests that PI3K inhibitors may be worth exploring as a potential therapeutic strategy for these tumors. However, the precise signalling pathways promoting tumorigenesis in this context need further exploration and the extent of Pten loss in human spinal tumors needs confirmation in larger studies [298].

Perhaps a complementary way of tackling gliomas is to determine how the proteins expressed on the cell surface of tumor cells differ from those of normal cells; this may allow us to define rationale targets on cancer cells for designing destructive therapeutic agents, such as antibodies or CAR-T cells, which would leave our normal cells alone and therefore potentially have few systemic side effects. In any case, it is almost certain that durable remissions of glioblastoma and other gliomas will only come about through multiple, complementary therapies that have been rationally based on molecular profiles of this cancer.