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Review

In vitro models of medulloblastoma: Choosing the right tool for the job

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ABSTRACT

The recently-defined four molecular subgroups of medulloblastoma have required updating of our understanding of *in vitro* models to include molecular classification and risk stratification features from clinical practice. This review seeks to build a more comprehensive picture of the *in vitro* systems available for modelling medulloblastoma.

The subtype classification and molecular characterisation for over 40 medulloblastoma cell-lines has been compiled, making it possible to identify the strengths and weaknesses in current model systems. Less than half (18/44) of established medulloblastoma cell-lines have been subgrouped. The majority of the subgrouped cell-lines (11/18) are Group 3 with *MYC*-amplification. SHH cell-lines are the next most common (4/18), half of which exhibit *TP53* mutation. WNT and Group 4 subgroups, accounting for 50% of patients, remain underrepresented with 1 and 2 cell-lines respectively.

In vitro modelling relies not only on incorporating appropriate tumour cells, but also on using systems with the relevant tissue architecture and phenotype as well as normal tissues. Novel ways of improving the clinical relevance of *in vitro* models are reviewed, focusing on 3D cell culture, extracellular matrix, co-cultures with normal cells and organotypic slices. This paper champions the establishment of a collaborative online-database and linked cell-bank to catalyse preclinical medulloblastoma research.

Contents

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1. Introduction

After accidents, childhood cancers are the most common cause of death in children older than 1 year in the developed world (Ward et al., 2014; WHO, 2015). On the global scale around 300,000 children and adolescents are diagnosed with cancer per year (Ferlay et al., 2013). Because paediatric malignancies occur early in life, they result in a tremendous loss of quality-adjusted-life-years both from death and from poor quality of life, due to the disabling consequences of treatment in surviving patients. That is why this group of childhood diseases has a profound effect on children themselves, their family, the healthcare system and society as a whole.

While leukaemia is the commonest cancer in childhood accounting for 30% of paediatric malignancies, brain and central nervous system (CNS) tumours are the most frequent in the solid tumour group making up 20% of childhood cancers (Ward et al., 2014). The most common paediatric malignant brain tumour is medulloblastoma, which is an embryonal tumour of the cerebellum. Medulloblastoma incidence rates peak at 6 children per million under 9 years of age and tail off to under 2 cases per million in the 15–19 age group (Ostrom et al., 2015). Overall, there are around 400 cases of medulloblastoma diagnosed in the US, 80 in the UK and an estimated 5000–8000 cases per year worldwide (Ferlay et al., 2013; Ostrom et al., 2015).

Current treatment for medulloblastoma combines surgery, radiotherapy and chemotherapy. This multimodal approach has transformed medulloblastoma from a virtually incurable disease in the beginning of the 20th century, to a manageable condition where 70% of children survive over 5 years and 58% for more than 15 years (Ward et al., 2014).

The increase in survival comes at the expense of serious and often long-lasting side effects. Medulloblastoma surgery carries a 25% risk of cerebellar mutism syndrome (Robertson et al., 2006), consisting of temporary total loss of speech, reduced co-ordination and balance. Although children eventually recover, they are left with lifelong defects in speed of processing, affecting all motor and cognitive functions. After surgery, adjuvant craniospinal radiotherapy is used to treat the whole brain and spine in medulloblastoma in order to prevent medulloblastoma dissemination in the cerebrospinal fluid (CSF). While craniospinal radiotherapy undoubtedly prolongs survival, it does so at the expense of 2–4 point IQ decrease per year (Mulhern et al., 2005) due to radiation-induced brain injury. Radiation has greater consequences for younger patients and is therefore contraindicated in children less than 3 years of age. Other toxicities from radiotherapy include impaired spinal growth and endocrine dysfunction. Together these side effects lead to reduced independence for patients and premature ageing (Boman et al., 2013). Despite hopes that proton therapy would have a move favourable side-effects profile by offering highly conformal dosimetry whilst sparing organs outside the brain, this treatment still requires whole brain and spine dosing with predictably similar CNS consequences (Yock et al., 2016). Therefore, the only way to reduce the incidence and severity of radiotherapy-induced side effects on the brain is to lower the radiation dose in those patients at reduced risk of tumour recurrence and dissemination and offer alternative adjuvant treatments. Chemotherapy, as the second adjuvant cancer treatment, offers this opportunity. Optimising techniques for the selection of chemotherapeutic agents targeted at the molecular subgroups of medulloblastoma and their application is the focus of this review.

Medulloblastomas are generally considered sensitive to conventional chemotherapy agents. Tumours exhibit objective responses, and patients on chemotherapy have increased event-free survival (Taylor et al., 2003). Disappointingly, a recent Cochrane review could not find evidence of chemotherapy improving overall survival in randomized clinical trials comparing regimens with and without chemotherapy (Michiels et al., 2015). Nevertheless, individual chemotherapy studies have reported 5-year overall survival rates greater than 80% in low-risk patients, without metastatic disease at diagnosis and complete resections (Packer et al., 1999). Similarly in a subset of desmoplastic medulloblastoma in infants, chemotherapy-only regimens have been reported to result in 5-year overall survival rates of 80% in patients younger than 3 years, in whom irradiation is contraindicated (von Bueren et al., 2011). The uncertainties of the contribution of chemotherapy to overall survival come from the empirical introduction of chemotherapy protocols to clinical practice and the lack of rational methods to predict individual patient response. Historically, chemotherapy clinical trials in medulloblastoma have been initiated based on anecdotal evidence and manageable side-effect profiles, rather than tumour target expression and drug-target engagement evidence.

The current challenge in chemotherapy is to identify which of the conventional chemotherapy agents and which new targeted drugs might be expected to work best in each of the new molecular subtypes of medulloblastoma. The constraints of low numbers of eligible patients and the significant costs and time involved for clinical trials necessitates a systematic preclinical screening strategy in order to select the best agents for the clinic. A rational approach to improving medulloblastoma therapy would rely on consecutive *in vitro* studies demonstrating intrinsic tumour sensitivity and lack of toxicity, followed by *in vivo* experiments documenting achievable tumour exposure levels and response to therapy. While preclinical data may not be able to predict patient response, they can increase the confidence in novel drugs through elucidation of mechanisms of action and resistance (Cook et al., 2014). Preclinical data can also be used to calculate theoretical exposures for tumour response and subsequently prioritise treatment strategies with the highest chance of success in the clinic. In this respect it is vital to link the data acquired in preclinical models to tumour subtypes

in order to identify patients who are most likely to benefit from a certain treatment.

The aim of this review is to systematise the available evidence on *in vitro* models of medulloblastoma and allow researchers to make informed choices on the usefulness of certain cell-lines, culture conditions and experimental setups. As medulloblastoma is no longer viewed as one disease, *in vitro* models that are representative of patient molecular subtypes and can be used to stratify patients for subgroup-specific therapies are essential.

2. The current classification of medulloblastoma

2.1. Histology

According to the old 2007 WHO classification, medulloblastoma was divided into four groups based on their histopathological features: classical, desmoplastic, medulloblastoma with extensive nodularity (MBEN) and large/cell anaplastic (Louis et al., 2007). While histology has been historically important in identifying high-risk patients (large-cell anaplastic) or those with favourable prognosis (infants with desmoplastic or MBEN morphology), it fails to stratify the majority of patients with classical histology.

2.2. Molecular subgroups of medulloblastoma

Extensive genomic studies have shown that medulloblastoma is not a single entity but is comprised of four distinct molecular groups (Taylor et al., 2012). The molecular subgroups have different genetic, epigenetic and phenotypic profiles and can predict patient risk and outcome. They are included in the latest 2016 WHO brain tumour classification as an additional "layer" alongside histology. Recent publications have revealed a complex risk-stratification subdivision within the molecular subgroups, identifying a number of smaller groups within the big four with better or worse prognosis (Ramaswamy et al., 2016; Shih et al., 2014).

2.2.1. WNT

Around 10% of medulloblastoma tumours exhibit aberrant WNT signalling with the majority of mutations in exon 3 of *CTNNB1*, nuclear expression of β-catenin and loss of chromosome 6. This group carries the best prognosis with over 95% of patients surviving over 5 years (Ellison et al., 2005). Recent studies have reported that patients with WNT-driven medulloblastoma have leaky blood vessels lacking the markers associated with an intact blood-brain barrier(Phoenix et al., 2016). These findings, suggesting potential for better drug access, may explain the increased chemosensitivity of WNT tumours and the favourable prognosis for patients.

2.2.2. SHH

The sonic hedgehog (SHH) pathway is perturbed in 30% of medulloblastoma tumours. This group combines patients with mutations in any of the factors in the SHH signalling pathway (PTCH1, SMO, SUFU, GLI1, and GLI2), leading to heterogeneity in disease presentation, response to therapy and outcome. While 5-year survival in the SHH group as a whole is around 64%, patients with either metastatic disease, *MYCN*, *GLI2* amplifications or chromosome 14q loss (Shih et al., 2014) and especially those with mutations in TP53 (Ramaswamy et al., 2016) have a very poor prognosis (<50% survival).

2.2.3. Group 3

Group 3 tumours comprise around 20% of medulloblastoma cases often present with metastatic disease at diagnosis and lead to relapse. This group carries the worst prognosis with less than 50% 5-year survival. While Group 3 has often been labelled as the MYC subgroup (Hatten and Roussel, 2011), MYC expression (mRNA or protein) is not prognostic and cannot inform subgroup classification. Since MYC is a downstream target of WNT signalling (Roussel and Robinson, 2013), MYC expression is also seen in the favourable WNT group. Superior prognostic high-risk factors within this group are *MYC* amplifications, seen in 17% of Group 3 tumours (3–4% of medulloblastoma patients as a whole) or isochromosome 17q (iso17q) which separately carry a poor prognosis (Shih et al., 2014).

2.2.4. Group 4

Group 4 tumours comprise the largest group (40% of patients). They occur predominantly in boys, carry an intermediate prognosis (70%, 5-year survival) and often exhibit iso17q. Although iso17q is also seen in Group 3 tumours (26%) it is much more common (66%) in Group 4 tumours (Northcott et al., 2011). While Group 3 and 4 tumours share other chromosomal abnormalities (e.g. gain of chromosome 18 and loss of 11p) and have similar gene expression features (e.g. *OTX2*, *FOXG1B*) they are molecularly distinct (Northcott et al., 2011). Prognostic factors within the group are chr11 loss or chr17 gain which were reported to be associated with favourable prognosis (Shih et al., 2014). A recent study has identified the LIM homeobox transcription factor 1 (LMX1A) as a major regulatory factor in Group 4 medulloblastoma, implicating progenitors from the upper rhombic lip as the cells-of-origin for Group 4 tumours (Lin et al., 2016). Nevertheless, Group 4 continues to be the largest and least understood subgroup of medulloblastoma.

2.3. Immunohistochemistry and other techniques for subgroup classification

Regarding immunohistochemistry, Northcott et al., 2011 advocated an affordable four-antibody approach to classify medulloblastoma tumours to the four molecular subgroups. However, subsequent studies have only been able to classify tumours into WNT, SHH and a mixed Group3/4 using this approach (Bien-Willner et al., 2012; Kaur et al., 2015; Min et al., 2013). This failure has been attributed to the poor ability of the NPR3 and KCNA antibodies to differentiate between Groups 3 and 4. That is why the current gold standard for subgroup profiling remains a choice between whole genome transcriptional array profiling (Taylor et al., 2012), employing a Nanostring panel of 22 genes (Northcott et al., 2012b) or methylation profiling (Schwalbe et al., 2013). A subgroup classification algorithm combining complimentary techniques has been suggested by (Gottardo et al., 2014). Additionally, a panel of fluorescence in situ hybridization (FISH) tests (GLI2, MYC, 14q, 17p, 17q, and 11q) has been promoted as a "pathology-friendly" technique for risk stratification (Shih et al., 2014).

3. *In vitro* models of medulloblastoma tumours

In vitro laboratory experiments on primary tissue and established cell-lines are routinely employed to test biological hypotheses and prioritise treatment strategies in cancer. While taking a tumour out of the body and placing it in an artificial mix of nutrients is a significant departure from the *in vivo* situation, the advantage of *in vitro* methods stems from the possibility of employing human patient-derived tissue, expanding the cells and using them in a broad range of experiments that are both inexpensive and yield rapid results.

3.1. Primary medulloblastoma cultures

The initial *in vitro* culture of cells and tissues harvested from patients or animals is deemed primary culture. While, primary cultures bear the highest resemblance to the *in vivo* state, they are often a heterogeneous mix of different cells. This makes them hard to standardise and frequently leads to increased variability

and findings which are difficult to reproduce by others. After the first passage, primary cultures are classed as early-passage cultures, where the cells are increasingly put under selective pressure in the cell culture media. Eventually, after a number of passages the cells either stop dividing (finite cell-lines) or they continue to divide forming continuous cell-lines. A key advantage of primary and early-passage cultures over long-established cell-lines is that they can be readily compared to the original tissue and the degree of similarity can be quantified, whilst long-established cell-lines may have adapted to *in vitro* growth conditions and the original patient tissue is rarely available.

Alternatively patient-derived tumour cells can be cultured and passaged in immunocompromised mice, preferably orthotopically, in the natural anatomical location (Shu et al., 2008). The theoretical advantages of this approach are that cells are not subjected to *in vitro* selection pressure of artificial media and that tumour cells are cultured in a more physiologically-relevant microenvironment. Although the introduction of tumour cells in mouse cerebellum has been historically challenging, Shu et al., 2008 have shown that tumour tissue from a single patient can be expanded into 200–2000 mice with 50% success rate and serially-passaged for up to 5 passages. This approach not only allows for expanding the tissue from one patient into multiple *in vivo* models, but the patient-derived tumours can later be cultured *in vitro* and subjected to high-throughput screens (Onion et al., 2016). Nevertheless, systematic genotyping and gene expression studies (Shu et al., 2008; Zhao et al., 2012) have indicated differences between patients and primary orthotopic xenograft cultures in 69 genes, involved in RAN, STAT and TP53 signalling (Fig. 2 and Supplementary Fig. S2a in (Zhao et al., 2012)). Furthermore, subsequent passaging *in vivo* was shown in the same studies to introduce genetic drift (up to 30 gene differences between passage 1 and 3) and loss of heterozygosity. The underlying reasons for these differences could be the mechanical and enzymatic dissociation procedures, the replacement of human with mouse stroma or selection bias for aggressive subclones from the patient's tumours. Therefore, despite differences between the two modalities, both *in vitro* and *in vivo* passaging introduce selection pressure and genetic drift.

3.2. Established cell-lines

Long-established continuous cell-lines have the advantages of being easy to expand and relatively uniform, therefore showing less variability compared to primary cultures. Provided that the same experimental conditions are used, experiments with continuous cell-lines can be repeated by different researchers all over the world adding to the reproducibility of experimental findings. The main criticisms towards them are again focused on selection bias, phenotypic and genetic drift (Wenger et al., 2004).

Currently, there are around 44 continuous medulloblastoma cell-lines established over a period of four decades. Because of the overwhelming diversity in characterisation methods used over the years, it can be very hard to see the full picture of the medulloblastoma cell-line landscape. Moreover, with the current molecular classification of medulloblastoma, it is vital to ascertain the subtype each cell-line belongs to in order to link preclinical data to patients' tumours. Two previous reviews have attempted to systematize the most common medulloblastoma cell-lines (Langdon et al., 2006) and include information on their subgroup affiliation (Xu et al., 2015a). This review builds on their work by expanding the number of classified cell-lines and including specific references with regards to the strength of evidence behind the classification (Table 1 and Fig. 1).

There is surprisingly little diversity in the genetic features for the large number of medulloblastoma cell-lines (Table 1 and Fig. 1). For example all Group 3 cell-lines harbour *MYC* amplification and 50% of the classified SHH cell-lines (UW-228 and DAOY) have a mutation in *TP53*. Indeed, even in the case of wild-type T*P53* cell-lines, TP53 function can also be supressed via MDM2 overexpression (Ghassemifar and Mendrysa, 2012; Kunkele et al., 2012). Both *MYC* amplifications and functional inactivation of TP53 are characteristics of high-risk aggressive medulloblastoma. This may mean that the more aggressive subtypes of medulloblastoma are generally better suited to grow *in vitro* and even in cases where more aggressive cells represent a minority population within the patient's tumour, the latter are selected and enriched through *in vitro* culture.

The early-passage MED6 cell line is most probably an example of *in-vitro* selection. Whilst the parent tumour harboured a mutation in codon 37 of CTNNB1, the MED6 cell-line exhibited a wild-type sequence for the gene (Othman et al., 2014). These results suggest that, while beta-catenin mutated cells were the dominating population in the parent tumour, a subpopulation of cells with wild-type beta-catenin were selected by culturing the cells *in vitro*.

The matched original tumour and metastasis cell-lines, D425 and D458 respectively, present an intriguing case because the cell-line derived from the original tumour (D425) is *TP53* mutated, while the one derived from the metastasis (D458) is *TP53* wild type (Ghassemifar and Mendrysa, 2012; Lacroix et al., 2014). While both possess the same R72P polymorphism, it appears that the *TP53* mutation was not necessary for the propagation of the metastatic cell-line. What makes this especially remarkable is the recent report that MYC and TP53 inactivation cooperate in medulloblastoma relapse in patients (Hill et al., 2015). The lack of information regarding the P53 status of the original biopsies makes it hard to establish whether the D425–D458 case is a real biological phenomenon or an artefact of cell culture.

The long-established D283 cell-line presents another curiosity, as it has been classified as either Group 4 (Snuderl et al., 2013) or Group 3 (Sengupta et al., 2014; Weeraratne et al., 2012). Therefore, the D283 cell-line, along with D721 and the newly established USP-13-MED, have been placed in an intermediate group between Groups 3 and 4. Both D283 and D721 show MYC overexpression at the mRNA and protein level and all three cell-lines exhibit OTX2 overexpression consistent with Group3/4 (Table 1). The uncertainties of classifying these cell lines to either Group 3 or 4 are most probably exacerbated by the similarities between the two groups (Ramaswamy et al., 2016).

While SHH and Group 3 tumours are extensively represented among cell-lines, there is a scarcity of *in vitro* models for Group 4 and especially WNT tumours. Until recently there had been no reports of WNT cell-lines in culture. However Othman et al., 2014 reported a medulloblastoma cell-line (MED5R) with β-catenin mutation derived from a rare case of recurrent large-cell anaplastic WNT tumour. Similarly, there is only one pair of cell-lines (CHLA-01-MED and CHLA-01R-MED), both derived from the same patient, which have been classified as Group 4. This is intriguing as over 40% of medulloblastoma tumours belong to this subgroup.

Finally, over half of the available cell-lines have not been subtyped or characterised to the standards of the molecular era of medulloblastoma research. The usefulness of the past research with these cells and any future experiments would greatly benefit from fuller characterisation and subtype classification using methylation or gene expression.

Table 2 shows a list of misidentified cell-lines and duplicate cell-lines which are either clones or refer to another cell-line. For example, TE-671 was the first medulloblastoma cell-line to be established (McAllister et al., 1977). Despite its initial widespread use it was subsequently discovered to be a clone of the rhabdomyosarcoma cell-line RD (Stratton et al., 1989). Similarly the initially reported human VC312R cell-line was later reported to be contaminated with mouse cells (Higgins et al., 2010; Parker and Pilkington, 2006). These two cases of misidentification are

Table 1
Systematic assessment table of all available continuous medulloblastoma cell-lines arranged according to subtype and molecular characteristics. Evidence for subgroup affiliation is classified as **Weak** in cases of conflicting reports and inconclusive data pointing to more than one subgroup; **Moderate**- evidence based on a single marker with no transcriptional profiling; **Strong**-transcriptional profiling data available and cell-lines classified to subgroups based on array data compared to patient tumours. The first references to mention each cell-line are referenced as superscript 1, references for specific claims are given as sequential superscript numbers. Abbreviations: M or mut.- mutated; wt-wild-type; WNT-wingless-related integrated site; CTNNB1- catenin-beta-1(beta-catenin); SHH-sonic hedgehog; NS-Nanostring; LCA-large-cell anaplastic; MYC-v-myc avian myelocytomatosis viral oncogene homolog; MYCN-v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog; OTX2-orthodenticle homeobox 2; PVT-1- plasmacytoma variant translocation 1 oncogene.

Cell-line	Subtype	*P53*	Evidence	Strength	Other features	References
MED5R[1]	WNT[1]		*CTNNB1* mutation[1] nuclear β-catenin IHC[1]	Moderate	recurrent WNT with LCA histology[1]	[1](Othman, 2014)
DAOY[1]	SHH[2]	M[3]	22 gene panel Nanostring (NS)[2]	Strong	Tetraploid	[1](Jacobsen et al., 1985) [2](Triscott et al., 2013);[3](Saylors et al., 1991), (Lacroix et al., 2014)
UW228[1] (3 cell lines same patient)	SHH[2]	M[3]	22 gene panel NS[2]	Strong		[1](Keles et al., 1995);[2](Triscott et al., 2013) [3](Kunkele et al., 2012; Lacroix et al., 2014)
UW426[1]	SHH[2]	–	22 gene panel NS[2]	Strong		[1](Akiyama et al., 2001; Yokota et al., 2004);[2](Triscott et al., 2013)
ONS-76[1]	SHH[2]	Wt[3]	22 gene panel NS[2]	Strong		[1](Yamada et al., 1989);[2](Triscott et al., 2013) [3](Kunkele et al., 2012; Lacroix et al., 2014)
D341[1]	Group 3[2]	Wt[3]	*MYC* amplification[1] Transcriptional profile[2]	Strong		[1](Friedman et al., 1988);[2](Weeraratne et al., 2012) [3](Kunkele et al., 2012; Saylors et al., 1991)
D384[1]	Group 3[2]	Wt[3]	*MYC* amplification[1] Transcriptional profile[2]	Strong		[1](Bigner et al., 1990; He et al., 1991);[2](Weeraratne et al., 2012) [3](Ghassemifar and Mendrysa, 2012)
D425[1] D458[2] (recurrence from same patient)	Group 3[3]	M[4] Wt[4]	*MYC* amplification[1] Transcriptional profile[3]	Strong	Diploid Tetraploid	[1](Bigner et al., 1990); [2](He et al., 1991) [3](Weeraratne et al., 2012) [4](Ghassemifar and Mendrysa, 2012; Lacroix et al., 2014)
D487[1]	Group 3	–	*MYC* amplification[2] *OTX2* amplification[2]	Moderate		[1](Bigner et al., 1997; Strickland et al., 1995) [2](Boon et al., 2005; Siu et al., 2003)
D556[1]	Group 3[2]	Wt[3]	*MYC* amplification[2]	Moderate		[1](Aldosari et al., 2002, 2000) mentioned by(Strickland et al., 1995) [2](Langdon et al., 2006);[3](Petersen et al., 2014; Waye et al., 2015)
MED8A[1]	Group 3[2]	Wt[3]	*PVT1-MYC* fusion[2] *MYC* amplification[4]	Strong		[1]T. Pietsch unpublished data; earliest publication(Lindsey, 2003) [2](Northcott et al., 2012a);[3](Lacroix et al., 2014);[4](Langdon et al., 2006)
MHH-MED-2[1]	Group 3	Wt[1]	*MYC* amplification[1]	Weak		[1](Pietsch et al., 1994);
MB002[1] MB004[1]	Group 3[1]	–	Gene expression panel[1] *MYC* amplification[1]	Strong		[1](Bandopadhayay et al., 2014)
HD-MB03[1]	Group 3[1]	Wt[1]	*MYC* amplification[1] Transcriptional profile[1]	Strong		[1](Milde et al., 2012)
D283[1]	Group 4[2]/3[3]	Wt[4]	No *MYC* amplification[5] High-level gain[6] GABRA5 expression[3]	Weak		[1](Friedman et al., 1985); [2](Snuderl et al., 2013); [3](Sengupta et al., 2014);(Weeraratne et al., 2012) [4](Saylors et al., 1991) [5](Bigner et al., 1990); [6](Langdon et al., 2006; Siu et al., 2003)
D721MED[1]	Group 4/3	–	2 *MYC* copies[2] High MYC expression[2]		High OTX2 mRNA[3,4] Low OTX2 protein[4]	[1](Aldosari et al., 2002, 2000; Bigner et al., 1997) [2](Siu et al., 2003);[3](Di et al., 2005);[4](Boon et al., 2005)
USP-13-MED[1]	Group 4/3 [1]	Wt[1]	*OTX2* gain[1]	Weak[1]		[1](Silva et al., 2015)

CHLA-01-MED[1] CHLA-01RMED[1] (matched recurrence)	Group 4[1]	Wt[1]	31-gene TaqMan Low Density Array[1] *MYC* amplification[1]	Strong	Myogenic differentiation[1] *BRCA2* 6174delT mut[1]	[1](Xu et al., 2015b)
MED3[1]	non-WNT[1]	–	wt *CTNNB1*[1]	Weak	normal *MYC*[1]	[1](Othman et al., 2014)
MED4 and MED4R[1] (matched recurrence)	non-WNT[1]	–	wt *CTNNB1*[1]	Weak	*MYCN* gain[1]	[1](Othman et al., 2014)
MED6[1]	WNT tumour non-WNT cell-line[1]	–	*CTNNB1* mut in tumour, but wt in cell-line[1]	Weak	normal *MYC*[1]	[1](Othman et al., 2014)
D581MED[1]	–	–	2 *MYC* copies[2] No MYC expression[2]		Low OTX2 mRNA[3,4] High OTX2 protein[4] Many chromosomal abnormalities[5]	[1](Strickland et al., 1995) [2](Siu et al., 2003) [3](Di et al., 2005) [4](Boon et al., 2005) [5](Aldosari et al., 2002)
D690MED[1]	–	–	–	–	–	[1](Aldosari et al., 2002)
MHH-MED-1[1]	–	Wt[1]	2 *MYC* copies[2]	–		[1](Pietsch et al., 1994);[2](Siu et al., 2003)
MHH-MED-3[1]	–	Wt[1]	–	–		[1](Pietsch et al., 1994)
MHH-MED-4[1]	–	Wt[1]	2 *MYC* copies[2]	–		[1](Pietsch et al., 1994);[2](Siu et al., 2003)
RES300[1]	–	–	–	–	–	[1](Sikkema et al., 2012)
Res 262[1]	–	–	–	–		[1](Kongkham et al., 2008)
Res 256[1]	–	–	–	–		[1](Bobola, 2005)
UW 473[1]	–	–	–	–	–	[1](Bobola, 2005)
UW 443[1]	–	–	–	–		[1](Keles et al., 1995);
UW 402[1]	–	–	–	–		[1](Castro-Gamero et al., 2013)
CHLA-259[1]	–	Wt[1]			High MYCN[1]	[1](Xu et al., 2012)
MCD-1[1]	–	M[1]				[1](Moore et al., 1996)
1580WÜ[1]					MMR defficient[1]	[1](Hartmann et al., 2005; von Bueren et al., 2012)
DEV[1]	–	–			Used as a model of differentiation[2]	[1](Giraudon et al., 1993; Jennings et al., 1992) [2](Buzanska et al., 2001)
IPNN-8[1]	–	–				[1](Rooprai et al., 1997)
Madsen[1]	–	–				[1](Ranger et al., 2010)
ONS-81	–	–				[1](Yamada et al., 1989)
BO-101						myomedulloblastoma (Giangaspero et al., 1991)

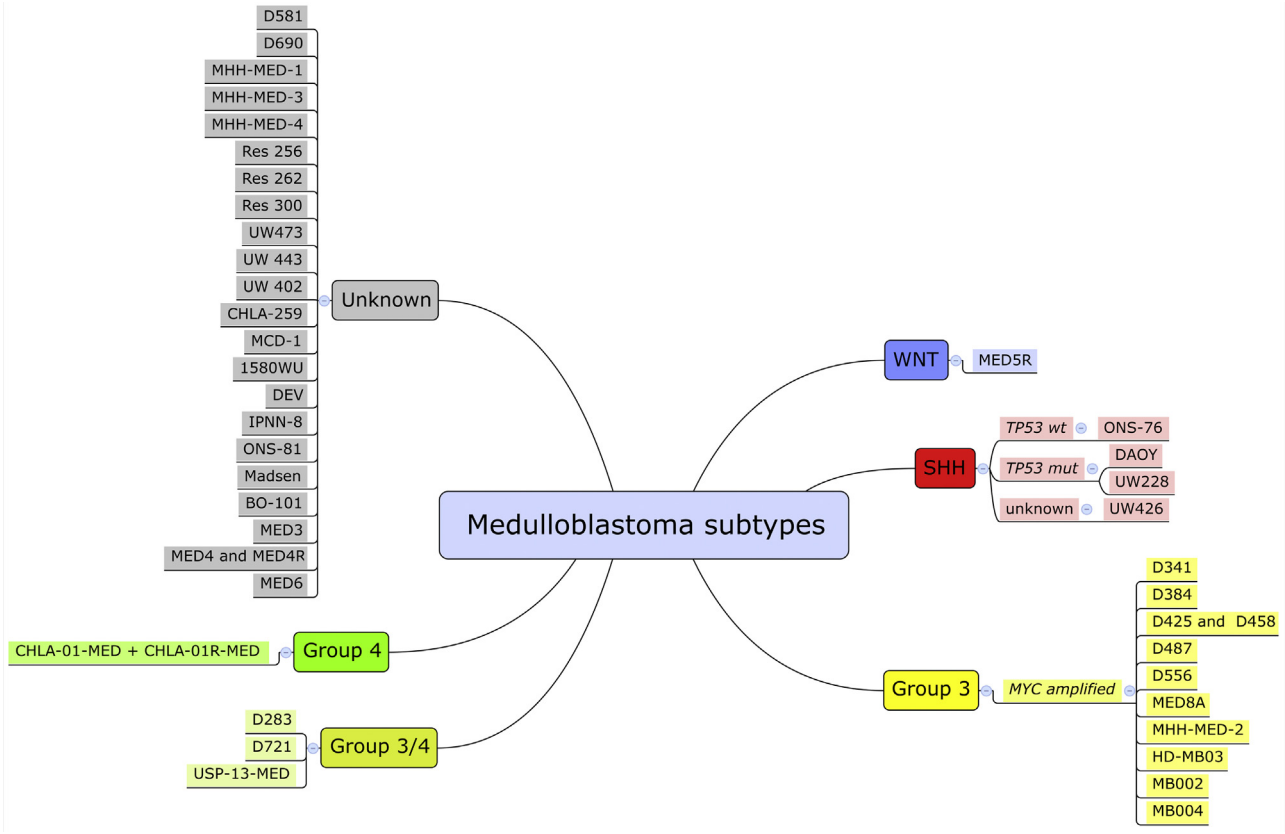

Fig 1. Organisational chart of medulloblastoma cell-lines classified by molecular subtype. WNT (blue); SHH-sonic hedgehog (red); Group 3 (yellow); Group3/4 (green-yellow); Group 4 (green); Unclassified (grey). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

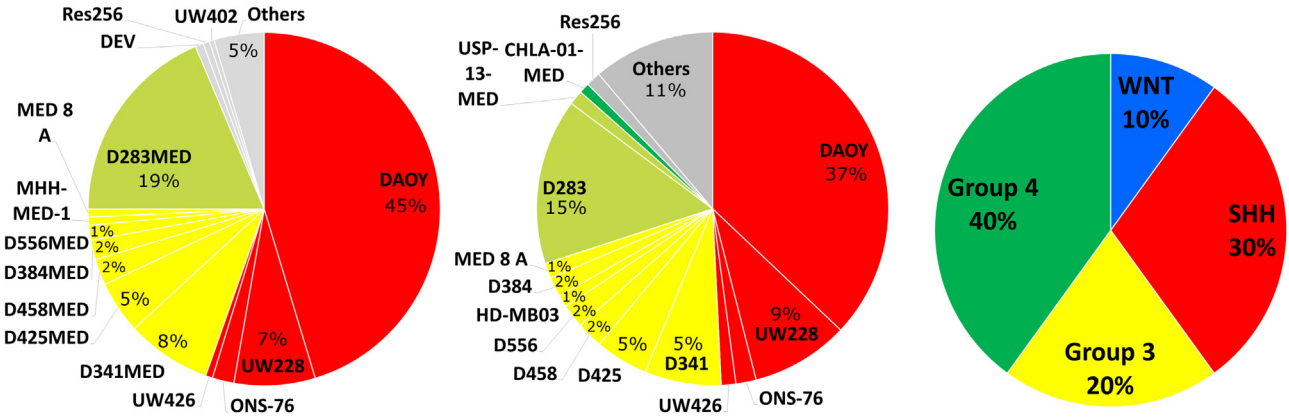

Fig. 2. Popularity of cell-line use calculated by number of citations. A-raw citations, B-citations normalised to the number of years the cell-line has been available; C-Frequency of medulloblastoma groups. Colours represent the various groups with WNT-blue, SHH-red, Group 3 yellow and Group 4 green. The D283 cell-line which has been classified as either Group 3 or 4 has been given a green-yellow colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Misidentified and duplicate cell-lines.

Cell-line	Reason for misidentification or duplication
ONS-B11 ONS-F11 ONS-F8	clones of ONS-76 (Sun et al., 2013)
D324[1] TE-671 VC312R	[1] synonym for DAOY (Cahan et al., 1994) [1] RD clone (Stratton et al., 1989) [1] mouse cell contamination (Higgins et al., 2010)

reminders of the importance of cell-line authentication (Coecke et al., 2005; Geraghty et al., 2014).

3.2.1. *Linking the data to patients – proportion of patient tumours covered*

A systematic literature search for the number of citations for each of the cell-lines from Table 1 revealed the most frequently-used cell-lines in medulloblastoma research (Fig. 2), the raw data and methodology are published in a linked Data in Brief article (Ivanov et al., 2016). Unsurprisingly the oldest cell-line DAOY (Jacobsen et al., 1985) was the most cited, closely followed by D283,

D341, UW2283, D425 and D458 (Fig. 2A). The DAOY hegemony in *in vitro* medulloblastoma research was not shaken even after accounting for the 31 years since the cell-line has been in culture (Fig. 2B). While the panel of SHH cell-lines is mentioned in around half of medulloblastoma *in vitro* experiments, the largest medulloblastoma subgroup- Group 4, represents around 1% of citations, even after dividing the citations by the number of years in circulation for each cell line (Fig. 2B). Fig. 2B and C illustrate the finding that more than 50% of patient tumours (when the number of patients with Group 4 and WNT tumours are combined) are not represented in preclinical medulloblastoma research. Furthermore, virtually all of the Group 3 model cell-lines harbour *MYC* amplifications (Fig. 1), a characteristic of only 17% of Group 3 tumours (Roussel and Robinson, 2013). Therefore in order to represent the full heterogeneity of patient tumours, there is an urgent need to establish and characterise more cell-line models of Group 4, WNT and Group 3 tumours (without *MYC* amplification) respectively.

3.2.2. GEMM cell-lines

Genetically engineered mouse models (GEMMs) of medulloblastoma have been derived with the aim of recapitulating medulloblastoma tumour development. Tumours in these models develop *de novo* in immunocompetent mice with natural tumour and stroma interactions. However, unlike medulloblastoma in humans, which exhibits four types of histology and metastasizes in 30% of patients, the majority of mouse models display classic histology, and only three models develop metastases- Smo/Smo (Hatton et al., 2008), Sleeping beauty (Wu et al., 2012) and GTML (Swartling et al., 2010). The vast majority of GEMMs require a Trp53 negative background, whereas *TP53* mutations are rare in medulloblastoma (10% of all patients), primarily seen in WNT (16% within group) and SHH (21% within group) subtypes (Zhukova et al., 2013). A recent paper (Pöschl et al., 2014) used agreement of gene expression analysis and k-means clustering to compare GEMMs to patient tumours and demonstrated that most GEMMs clustered in the SHH subgroup, one model with WNT and one (GTML) with Group 3. The *Trp53*-negative background of the *Myc*-driven models placed them somewhere between SHH and Group 3. Moreover, the SHH GEMMs clustered closer to adult SHH medulloblastoma than to tumours in infants and children. In this respect, GEMMs provide a useful platform to study *in vivo* phenomena, like increased blood-brain barrier permeability in WNT tumours (Phoenix et al., 2016), but do not currently offer alternative sources of cells representative of Group 4, *TP53* wt WNT or Group 3 patient tumours.

Nevertheless, high-throughput screens with GEMM derived-cells have been instrumental in projects aimed at repurposing existing chemotherapy agents for use in medulloblastoma (Morfouace et al., 2014). Employing them in the screening phase identified two promising FDA approved drugs, pemetrexed and gemcitabine, to take into *in vivo* studies. In subsequent experiments, the inclusion of pemetrexed and gemcitabine, alongside standard cisplatin and cyclophosphamide chemotherapy, increased survival in patient-derived xenograft models from 39 days for vehicle control animals to 63 days for the combined 4-drug regimen. These studies have resulted in a clinical trial evaluating the inclusion of gemcitabine and pemetrexed alongside standard chemotherapy for Group 3/4 patients (NCT01878617).

4. *In vitro* models of the normal brain

4.1. Reasons to include the normal brain

While testing drugs on medulloblastoma cell-lines and primary cultures provides clues about tumour drug sensitivity and potential efficacy of compounds, these experiments may select for inherently toxic compounds or exposure times. Therefore, normal tissue controls need to be included in early-stage screens in order to ascertain tumour selectivity and lack of normal tissue toxicity. Apart from the plethora of side effects systemic chemotherapy has on dividing tissues, such as the bone marrow and epithelial tissues, CNS-targeted chemotherapy always carries the risk of neurotoxicity. Cisplatin for example, notoriously damages the cochlea, causing hearing loss in children with medulloblastoma (Lafay-Cousin et al., 2013). Vincristine exhibits peripheral neurotoxicity when given via the systemic route, and carries the risk of fatal neurotoxicity if administered intrathecally (Alcaraz et al., 2002). Neurotoxic side-effects are especially important for local drug delivery strategies aimed at controlling leptomeningeal metastasis. Intra-cerebrospinal fluid administration of methotrexate is known to cause leukoencephalopathy (Bhojwani et al., 2014) and liposomal cytarabine requires concomitant dexamethasone to control chemical arachnoiditis symptoms (Chamberlain, 2012). Therefore, comparison of the toxicity of therapy towards normal brain tissue is required from the earliest stage of drug development in addition to the standard panel of *in vitro* safety assessments. The use of normal tissue can help define the therapeutic window of chemotherapy necessary to eliminate the tumour without causing major damage to the brain.

4.2. Rodent neural stem cells

Mouse and rat foetal brain cultures have demonstrated the ability to correctly identify neurotoxic compounds (Hayess et al., 2013; Zurich et al., 2013). However, recent studies have shown important interspecies differences in chemical and drug neurotoxicity towards rodents and humans (Baumann et al., 2015). The long-standing concerns about the ability of animal cells to predict response in humans (Abbott et al., 1999; Leist and Hartung, 2013), have underpinned the use of human tissues for neurotoxicity assessment.

4.3. Transformed human cells

The most easily accessible types of “normal” human cells are the transformed human cell-lines. They have been derived from healthy donors, are relatively stable, easy to expand and can be differentiated to neurons and glia. The most commonly used examples are induced pluripotent stem cells (iPS), the LUHMES cell-line and the commercially available ReNCell lines (Hoffrogge et al., 2006; Lotharius et al., 2002; Schwartz et al., 2015; Takahashi et al., 2007). While these cell-lines can be used to inform research into neurodevelopmental disorders and neurodegenerative diseases, their application as normal brain surrogates in brain tumour research is questionable. The transduction with SOX-2 (for iPS cells) or MYC (iPS, LUHMES, ReNCell) makes these cell types similar to SHH and Group 3 medulloblastoma tumours respectively. The oncogene transformation and the tumorigenicity of the transformed cell-lines, hinders their application as normal tissue surrogates and necessitates the use of non-transformed normal tissues.

4.4. Human neural stem cells

Non-transformed human neural stem cells can be derived from human umbilical cord blood stem cells (Bużańska et al., 2005), human embryonic stem cells (Chambers et al., 2009) or foetal brain tissue (Carpenter et al., 1999). One disadvantage of the former, less-differentiated subtypes is that the embryonic stem cells need to be cultured for a few weeks in long multistep protocols in order to reach the neural stem cell state (Chambers et al., 2009; Zhang et al., 2001). Moreover, there are substantial differences in gene expression patterns for neural stem cells derived from embryonic

stem cells and those derived from foetal brain (Shin et al., 2007). Foetal cultures display a more specialised, differentiated phenotype, while the embryonic-derived neural stem cells represent an earlier stage of development. Foetal brain tissue, on the other hand, is very heterogeneous and the state of differentiation and the number of achievable passages in culture depends on the gestational age of the foetus at the time of isolation (Moors et al., 2009). Regardless of the source, human neural stem cells need to be differentiated towards neurons, glia and oligodendrocytes in order to form reliable models of the human brain.

5. Three-dimensional models

5.1. Why 3D?

While the genetic makeup of medulloblastoma and neural cells is essential for determining their suitability in representing patient tumours and the normal brain, *in vitro* culture conditions can influence the phenotype, cell signalling and drug sensitivity to a large extent (Hickman et al., 2014). Simplistic monolayer cultures, where a single layer of cells is attached to polystyrene, have unnaturally low cell densities, lack cell–cell or cell-extracellular matrix interactions, and do not exhibit nutrient gradients or physiological levels of oxygen. Therefore, it is not surprising that when conventional monolayer cultures of medulloblastoma cell-lines are compared to patient tumours, the cell-lines tend to cluster together and, not with patient tumours of the same subtype (Lin et al., 2016).

5.2. Multicellular 3D tumour spheroids

Multicellular tumour spheroids, first popularised by Sutherland et al., recapitulate the physiological characteristics of tumour and normal tissues in a superior way, compared to monolayers. The majority of tumours and normal cells *in vivo* grow as three-dimensional tissues in contact with each other and the extracellular matrix, displaying a gradient in oxygen levels (2–5%) and nutrients, depending on their proximity to blood vessels. In this respect, cells cultured as spheroids can often synthesize their own extracellular matrix, mimicking natural cell–cell and cell-matrix interactions (Cukierman et al., 2001; Ekert et al., 2014). Moreover, small avascular tumour micrometastases frequently display a hypoxic core with quiescent cells which are more resistant to radiation and chemotherapy (Carrera et al., 2010; Herrmann et al., 2008; Rodríguez-Enríquez et al., 2008). In this respect, multicellular tumour spheroids can be cultured to sizes beyond the diffusion distance of oxygen (300–500 mm) and exhibit similar gradients and resistance patterns (Doublier et al., 2012; Wartenberg et al., 2003). Culturing cells in three-dimensional models also influences the stem cell compartment (Hussein et al., 2011) and drug sensitivity (Hussein et al., 2011; Smith et al., 2012). Finally, gene expression profiles in spheroids have been reported to be closer to those of parent tumours compared to monolayers (De Witt Hamer et al., 2008; Sakai et al., 2010).

5.3. Three-dimensional normal brain component

The normal cerebellum, adjacent to the tumour, is a complex three-dimensional structure composed of cerebellar nuclei, cerebellar granular neurons, Purkinje cells, stellate cells, basket cells, astrocytes, oligodendrocytes, microglia, ependymal cells covering the fourth ventricle, cells of the choroid plexus, etc. Therefore a realistic model of the normal human brain in the context of medulloblastoma would need to be a 3D structure with a complex network of differentiated neurons and glia along with some progenitor cells.

Human neurospheres fulfil most of the above conditions, albeit presenting as a simple mix of progenitors, glial and neuronal cells (Campos, 2004; Moors et al., 2009). Importantly, they have the potential to assess toxic effects on mixed cultures of neurons, astrocytes and oligodendrocytes taking into account the interactions between the cell types and their toxicological implications. For example glial cells can not only affect the differentiation of neurons (Wang et al., 1994), but can also convert toxic compounds to active metabolites (Ransom et al., 1987) or secrete apoptotic factors upon drug treatment (Wang et al., 2011). Although neurospheres are dominated by poorly-differentiated cells, these progenitors may model important collateral damage targets in children's brains. For example, insults to the hippocampal structures known to host progenitors, have been linked to neurocognitive deficits in medulloblastoma patients (Riggs et al., 2014).

The neurons and glial cells in children's brains are well-differentiated, have established connections and are characterised by an ongoing process of myelination, pruning and maturation. In this respect, the latest neurosphere models (Ivanov et al., 2014) are at a disadvantage in representing the child's brain due to their less-mature foetal phenotype. Making them more physiologically-representative requires differentiation and maturation protocols. Although some differentiation protocols have already been developed, they rely on reductionist monolayer cultures (Visan et al., 2012) or chemotherapy agents to stop proliferation (Jagtap et al., 2011; Smirnova et al., 2015). The cerebral organoid cultures developed by Lancaster et al., 2013 represent a major step in the modelling of the human brain *in vitro*. However, in order to be useful in chemical screens, future protocols need to be in high-throughput format and take days before the start of drug treatment, not months. Therefore, there is an urgent need to develop 3D *in vitro* models of the human brain with non-transformed cells in a high-throughput format and a short time to differentiation.

5.4. Spheroid co-cultures

Another vital aspect of modelling medulloblastoma is including the interaction between normal and tumour tissue. The interplay between tumour and host tissue has been repeatedly demonstrated to affect chemosensitivity (Straussman et al., 2012), radiosensitivity (Upreti et al., 2011), proliferation (Spink et al., 2006), angiogenesis (Wartenberg, 2001), cell adhesion (Chambers et al., 2011) and gene expression (Berg et al., 2014). Including a normal tissue component has proven to be essential in the successful development of lung cancer (Amann et al., 2014; Onion et al., 2016), bladder cancer (Kilani et al., 2003, 2002) as well as glioblastoma models (Chintala et al., 1997; Go et al., 1997; Terzis et al., 1997a, 1997b; Thorsen et al., 1997).The combined scientific evidence suggests that models incorporating a tumour and normal tissue component are more than the sum of their parts and exhibit increased physiological relevance. In view of these considerations the ultimate model of medulloblastoma is envisaged to be a co-culture model of normal and tumour tissue where the viability of each cell population can be determined separately (Ivanov et al., 2015), provided that the right differentiation state can be achieved.

5.5. ECM

In the context of the brain, the extracellular matrix is a subject that is often overlooked, despite constituting around 10–20% of the volume of the brain (Rauch, 2007). The ECM is an important component which affects cell signalling, drug distribution and metastasis (Bellail et al., 2004). The brain ECM consist mainly of glycosaminoglycans like chondroitin sulphate, hyaluronan, and complexes with lectican, aggrecan and link proteins (Rauch, 2007). An important consideration for *in vitro* models is the low percentage of collagens,

with mainly collagen IV in the basement membrane layers of the blood-brain barrier. The basement membrane is also the principal location where laminin and fibronectin are major ingredients of the brain ECM. In this respect, *in vitro* matrices composed of collagen I are physiologically-different from the brain parenchyma. Similarly the laminin-rich Engelbreth-Holm-Swarm (EHS) mouse sarcoma derived matrices (e.g. Matrigel) may be more appropriate for blood-brain barrier studies than modelling invasion throughout the brain. Therefore models incorporating these matrices (Deisboeck et al., 2001; Kumar et al., 2015) may give a mechanistic clue of invasion with regards to cell motility within gels, but extrapolation of the findings to the clinical setting may be limited. A potential way to overcome the above limitations is to use decellularised brain ECM preparations which have been reported from porcine (DeQuach et al., 2011) and murine sources (De Waele et al., 2015; Zhu et al., 2015). While these matrices have been derived from brain tissues and contain a brain-specific mix of glycosaminoglycans, interspecies differences in composition and pore size cannot be ignored. Other alternatives are synthesizing tuneable hydrogels and tailoring them to match ECM components (Ananthanarayanan et al., 2011; Karumbaiah et al., 2015). Adding to the complexity, metastasis and invasion in the brain is also influenced by the interaction of invading tumour cells with the normal neuronal and glial cells and their spatial arrangement.

5.6. *Organotypic* ex vivo *models with cerebellar slices*

In this respect, *ex vivo* cerebellar slices are the laboratory models which offer the closest representation of the *in vivo* state of the brain outside of the body (Lu et al., 2011). Organotypic brain slices have been derived from rodent pups (Stoppini et al., 1991) and adult human brain (Jung et al., 2002). They possess the *in vivo*-like architecture, mix of differentiated cells in the right spatial organisation and the native extracellular matrix of the brain. These advantages have stimulated their use in experiments modelling tumour invasion and metastasis in glioma (Aaberg-Jessen et al., 2013; Jung et al., 2002) as well as drug delivery in medulloblastoma (Meng et al., 2016, 2007). They have also been utilised to study the effects of chemotherapy on the normal brain (Nørregaard et al., 2012). However the increased biological relevance of these models comes at the price of low-throughput, demanding manual work to derive the cultures, limited life-span and variability between different slice preparations. Nevertheless organotypic cultures can serve as an important bridge between cell-based *in vitro* cultures and *in vivo* studies- reducing and refining the use of animals or taking advantage of leftover human brain tissue from epilepsy surgery (Eugène et al., 2014).

6. Data register/Cell-line database

This review has attempted to systematize all of the available cell-lines for medulloblastoma research according to cell type and published molecular characteristics in the literature. Half of medulloblastoma cell lines are poorly characterised and the data for the other half had to be extracted by piecing together small pieces of information from multiple papers. For example, the genomic profile of the second most commonly cited cell line, D283, has been uploaded many times to the NCBI's Gene Expression Omnibus (Edgar, 2002) (accession numbers GSE14437; GSE36133; GSM919357; GSM482336; GSE37412) by multiple authors (Barretina et al., 2012; Beroukhim et al., 2010; Dalwadi and Sunderland, 2008; Northcott et al., 2012a, 2009). Nevertheless, it has been affiliated with Group 4 (Snuderl et al., 2013), Group 3 (Sengupta et al., 2014; Weeraratne et al., 2012) and even WNT/SHH subtypes in some more controversial computer-classification approaches (Gendoo et al., 2015). The fragmented state of cell-line characterisation can result in research with cells which are not representative of patient tumours or using redundant panels of Group 3 cell lines e.g. all with *MYC*-amplification. This work has identified 23 medulloblastoma cell lines which would benefit from further characterisation and subtyping.

A future platform for medulloblastoma model selection should allow inclusion of the latest developments in the field and amendment of erroneous information from previous editions. Hence, we are proposing a collaborative project *via* an online database of medulloblastoma *in vitro* tools. The database would include authentication data (short tandem repeats), molecular classification, and omics-type data as well as drug sensitivity information for each cell line. It would enable individual researchers to upload data from authenticated cell lines cultured as monolayers or more physiologically-relevant *in vitro* and *in vivo* models and would facilitate comparisons with primary medulloblastoma tumours.

Another way of aiding researchers in choosing the right *in vitro* tools would be establishing a central medulloblastoma cell bank linked with the online database of all medulloblastoma cell lines. The cell bank would serve as a reference source for each cell-line allowing individual researchers to identify relevant cells to answer their individual research questions and to access well-characterised cells. The combination of cell bank and online data repository would assist in rapid recognition of the gaps in preclinical research in terms of representation of tumour types.

7. Conclusions and future directions

Since the establishment of the first medulloblastoma cell-lines, over four decades of *in vitro* medulloblastoma research has produced a wide variety of models to study medulloblastoma biology and test potential therapies. Despite a great deal of biological characterisation, one key piece of information is still missing. How good are these models in predicting patient response? The predictive potential of the current preclinical medulloblastoma models has not been determined in a concerted systematic way.

The prevailing belief is that matching the molecular circuitry of human tumours in the lab would yield better predictions. Nevertheless, the extent of complexity necessary for faithful predictions of biological behaviour remains unknown. Researchers have investigated similarity in the genetic, epigenetic, RNA, protein and metabolomics level without reaching consensus on what level of agreement is good enough. For example, despite the vast amount of *in vitro* and xenograft studies performed with the DAOY cell-line, most clinicians would be reluctant to base treatment on these data, even in cases of SHH tumours displaying the same molecular features as DAOY (SHH, tetraploid and *TP53* mutated).

In that respect, it is equally important to determine the added value of culturing cells in 3D, using the right ECM and including a normal brain component. Do interventions like these sufficiently improve the predictive potential of models? In order to place *in vitro* models in the clinical context, we would need to compare their level of physiological similarity to the latest *in vivo* models of medulloblastoma and, more importantly, patient tumours. Although agreement of gene expression analysis (AGDEX) and clustering approaches have been used to compare mRNA profiles of the latest GEMMs to patient tumours, no cell-lines or primary cells were included in that analysis (Pöschl et al., 2014). It would be beneficial to use the same methodology and compare the existing cell-lines in 2D and 3D against mouse models and patient tumours. This may allow the level of similarity necessary for faithful predictions of safety and efficacy to be uncovered.

This review has mainly been concerned with *in vitro* models which are often dismissed in the *in vivo* community as unreli-

able and overly simplistic. This is true when considering that most conventional *in vitro* models do not account for drug distribution, metabolism or excretion. However, interspecies differences are well-documented between human and rodents in blood-brain barrier permeability (Shen et al., 2004), metabolism and excretion (Leist and Hartung, 2013; Rangarajan and Weinberg, 2003; Singh and Ferrara, 2012). The above limitations make the extrapolation of all preclinical data to the clinic challenging. Nevertheless *in silico* approaches accounting for physiologically-based pharmacokinetic modelling and machine-learning algorithms can help in improving the predictive potential of preclinical studies (Schwartz et al., 2015; Yoon et al., 2012).

In addition to model considerations, predictions of drug activity are related to drug exposure- concentrations achieved at the target over a period of time. While the majority of *in vitro* experiments correlate drug concentration in media and maximum plasma levels, this approach has a number of downsides. Drugs in *in vitro* experiments can bind to albumin and ECM components in cell culture media, they can be pumped out of the cells or distribute in the wrong intracellular compartment (Groothuis et al., 2015). *In vivo,* blood, CSF or brain parenchyma concentrations may differ from intratumoral levels by several orders of magnitude (Muldoon et al., 2007). Studies correlating *in vitro* cell-target levels with *in vivo* intratumoural target engagement over time are needed for reliable predictions.

The full potential of *in vitro* models can be realized in prospective clinical trials, where tumour and normal tissue are collected from every patient, and short term *in vitro* cultures established during the course of treatment as in colorectal cancer, for example (van de Wetering et al., 2015). The intrinsic tumour and normal tissue sensitivity to clinically-relevant therapeutic interventions can be prospectively tested *in vitro* and compared to the outcomes of clinical treatment, allowing the construction of *in vitro-in vivo* correlation mathematical models. This endeavour should also combine genomic, transcriptomic and proteomic analysis to identify trends between molecular circuitry and drug sensitivity (Barretina et al., 2012; Garnett et al., 2012; Yang et al., 2013). The results can be extrapolated to other patients by computing similarity scores for *in vitro* models, including cell lines, based on genomic, proteomic or metabolomics data and predicting patient response through the established machine-learning algorithms, validated in prospective clinical studies.

The way forward for useful preclinical models is to move away from the binary measures of sensitivity and resistance and correlate target drug exposures over time with clinical response. In addition, clinical response should not be reduced solely to tumour response but would ideally relate to survival and quality of life improvement. The preclinical models of the future should always relate the acquired data to patients and interpret them in the context of the clinical situation.

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Appendix A. Supplementary data

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