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New targeted therapies for malignant neural tumors

From systematic discovery to zebrafish models

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Abstract
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Cancers in the neural system presents a major health challenge. The most aggressive brain tumor in adults, glioblastoma, has a median survival of 15 months and few therapeutic options. High-risk neuroblastoma, a childhood tumor originating in the sympathetic nervous system, has a 5-year survival under 50%, despite extensive therapy. Molecular characterization of these tumors has had some, but so far limited, clinical impact. In neuroblastoma, patients with *ALK* mutated tumors can benefit from treatment with ALK inhibitors. In glioblastoma, molecular subgroups have not yet revealed any subgroup-specific gene dependencies due to tumor heterogeneity and plasticity. In this thesis, we identify novel treatment candidates for neuroblastoma and glioblastoma.

In **paper I**, we discover novel drug targets for high-risk neuroblastoma by integrating patient data, large-scale pharmacogenomic profiles, and drug-protein interaction maps. Using a novel algorithm, TargetTranslator, we identify more than 80 targets for this patient group. Activation of cannabinoid receptor 2 (CNR2) or inhibition of mitogen-activated protein kinase 8 (MAPK8) reduces tumor growth in zebrafish and mice models of neuroblastoma, establishing TargetTranslator as a useful tool for target discovery in cancer.

In **paper II**, we screen approximately 1500 compounds across 100 molecularly characterized cell lines from patients to uncover heterogeneous responses to drugs in glioblastoma. We identify several connections between pathway activities and drug response. Sensitivity to proteasome inhibition is linked to oxidative stress response and p53 activity in cells, and can be predicted using a gene signature. We also discover sigma receptors as novel drug targets for glioblastoma and find a synergistic vulnerability in targeting cholesterol homeostasis.

In **paper III**, we systematically explore novel targets for glioblastoma using an siRNA screen. Downregulation of ZBTB16 decreases cell cycle-related proteins and transcripts in patient-derived glioblastoma cells. Using a zebrafish assay, we find that ZBTB16 promotes glioblastoma invasion *in vivo*.

In **paper IV,** we characterized the growth of seven patient-derived glioblastoma cell lines in orthotopic zebrafish xenografts. Using automated longitudinal imaging, we find that tumor engraftment strongly correlates with tumor initiation capacity in mice xenografts and that the heterogeneous response to proteasome inhibitors is maintained *in vivo*.

In summary, this thesis identifies novel targets for glioblastoma and neuroblastoma using systematic approaches. Treatment candidates are evaluated in novel zebrafish xenograft models that are developed for high-throughput glioblastoma and neuroblastoma drug evaluation. Together, this thesis provides promising evidence of new therapeutic options for malignant neural tumors.

Keywords: neuroblastoma, glioblastoma, data integration, zebrafish models, precision medicine

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To my family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Almstedt, E.,** Elgendy, R., Hekmati, N., Rosén, E., Wärn, C., Olsen, T.K., Dyberg, C., Doroszko, M., Larsson, I., Sundström, A., Arsenian Henriksson, M., Påhlman, S., Bexell, D., Vanlandewijck, M., Kogner, P., Jörnsten, R., Krona, C., Nelander, S. (2020) Integrative discovery of treatments for high-risk neuroblastoma. *Nature Communications*, 11(1):71.

II Johansson, P., **Almstedt, E.,** Doroszko, M., Kundu, S., Vinel, C., Schmidt, L., Baskaran, S., Lundsten, S., Rosén, E., Elgendy, R., Elfineh, L., Häggblad, M., Martens, U., Lundgren, B., Frigault, M.M., Lane, D.P., Nestor, M., Marino, S., Krona, C., Nelander, S. A drug association map of glioblastoma informs precision targeting of p53-dependent metabolic states. *Cell Reports, in revision.*

III Baskaran, S., **Almstedt, E.,** Hansson, C., Kalushkova, A., Atienza Párraga, A., Spyrou, A., Forsberg Nilsson, K., Jernberg Wiklund, H., Elfineh, L., Weishaupt, H., Kundu, S., Krona, C., Nelander, S. ZBTB16 orchestrates growth and invasion in glioblastoma. *Manuscript*

IV **Almstedt, E.,** Rosén, E., Gloger, M., Hekmati, N., Koltowska, K., Krona, C., Nelander, S. Real-time evaluation of glioblastoma treatments in patient-specific zebrafish xenografts. *Manuscript*

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Cancers

Cancer is one of the leading causes of death in the world with approximately 18 million new cases and 9.6 million deaths in 2018 (Bray et al., 2018). Comprising more than 100 separate entities, cancer diseases are usually classified by the primary site tissue or the cell-of-origin. Clinically, cancer is divided into different stages, from localized disease to invasive or metastatic disease, which in most cases is associated with a worse prognosis.

Cancer diseases are thought to be caused by genomic alterations (mutations), leading to unrestrained cell proliferation. The genetic alterations of cancer cells are observed at different levels, including point mutations, gene truncations, deletions, translocations, copy number alterations, overexpression, or by epigenetic regulation. Cancer-associated gene alterations can appear randomly during cell division, as the number of stem cell divisions required to form a tissue is associated with an increased risk of developing cancer in that organ (Tomasetti and Vogelstein, 2015), or by external stimuli, e.g. exposure to mutagens such as UV light or tobacco smoke (Alexandrov et al., 2013). Age is one of the leading risk factors for developing cancer, likely due to the accumulation of acquired mutations during life (Alexandrov et al., 2013). Generally, childhood tumors have a lower mutational burden than adult tumors (Alexandrov et al., 2013; Lawrence et al., 2013). In children, 7-8% of patients carry germline gene variants that predispose for cancer (Gröbner et al., 2018).

Cellular processes involved in cancer development and maintenance

The mutations that cause cancer affect several dimensions of cellular functions, sometimes referred to as Hallmarks of cancer (Figure 1) (Hanahan and Weinberg, 2011). First, cancer is characterized by uncontrolled proliferation as a result of oncogene activation, reduced ability to induce cell death, inactivation of tumor suppressor pathways, and replication immortality. Second, to sustain the elevated need of energy and oxygen as a result of cell growth and proliferation, cancer cells alter their metabolism and induce angiogenesis (Hanahan and Weinberg, 2011). Third, malignant cells commonly activate migratory and invasive pathways to invade into surrounding tissue. Forth, cancer

cells induce a proinflammatory microenvironment, which promotes tumor growth and lacks an effective anti-tumor immune response. Underlying the mutation diversity is an increased genomic instability, which leads to an increased mutation rate and intratumoral heterogeneity (Hanahan and Weinberg, 2011).

Cancer-associated genetic alterations are particularly frequent in a set of cellular pathways that regulate oncogenic signaling and such alterations are shared between many tumor types (Bailey et al., 2018; Sanchez-Vega et al., 2018). Commonly altered pathways include cell cycle regulators, such as the cyclin-dependent kinase (CDK) family of proteins, receptor tyrosine kinases (RTKs; e.g. ALK, and EGFR), and developmental transcription factors, such as Myc (Sanchez-Vega et al., 2018; Schaub et al., 2018). Activation of downstream signaling pathways, e.g. PI3K-AKT-mTOR and RAS-MAPK pathways, or alternatively, by the inactivation of pathway inhibitors, such as CDKN2A/B/C, Rb1, PTEN, and NF1, are also frequent in cancer (Sanchez-Vega et al., 2018). To avoid regulated cell death through apoptosis, tumor cells often inactivate the tumor suppressor p53 (Sanchez-Vega et al., 2018). During replication in normal somatic cells, telomeres are shortened, leading to a limited number of successive cell divisions for a cell. Replication immortality require activation of telomere maintenance pathways, typically by activating telomerase reverse transcriptase (TERT) or through the alternative lengthening of telomeres (ALT) pathway (Hanahan and Weinberg, 2011). Together, these alterations give a survival benefit for the cancer, but can also affect the cells negatively and induce cellular stress. To cope with this, the cell activates compensatory mechanisms, which can be exploited for therapies.

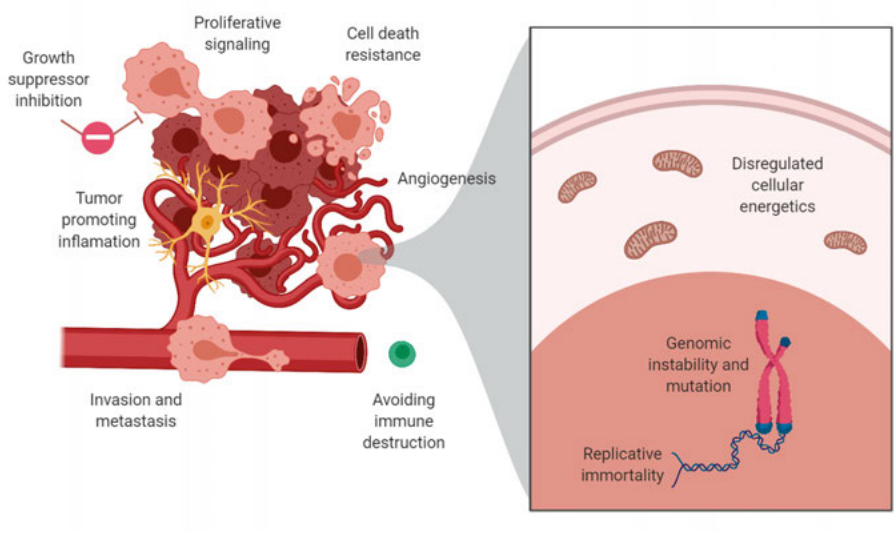

Figure 1. Hallmarks of cancer.

Treating cancer using conventional and targeted therapies

The current treatment regimen for most solid tumors includes a combination of surgery, radiotherapy, and chemotherapy. For some tumor types, there are additional treatment options. These include (i) stem cell transplantation or immune checkpoint blockade that boost the immune response against the tumor, e.g. PD1 inhibition in malignant melanoma (Robert et al., 2015), (ii) differentiation therapy to limit the proliferative capacity of the tumor cells, e.g. 13-*cis*-retinoic acid in neuroblastoma (Matthay et al., 1999, 2009), and (iii) targeted therapy by inhibiting key oncogenes in the tumor cells, e.g. HER2 inhibition in HER2-positive breast cancer (Ryan et al., 2008; Slamon et al., 2001). Other modes of therapy have shown promising effects in clinical trials, e.g. tumor treating fields in glioblastoma (Stupp et al., 2017), but have not yet made it into standard treatment regimens. To an increasing extent, treatments are tailored to every patient based on clinical or molecular biomarkers, a strategy often referred to as *precision medicine*. Two common strategies to evaluate precision therapy for cancer patients are umbrella trials (i.e. dissecting out all possible targets within one disease) and basket trials (i.e. targeting a common molecular alteration in several different diseases) (Hierro et al., 2019). The aim of precision medicine is to increase the treatment accuracy in a way that a patient receives the right treatment, at the right time, and with minimal effect on non-cancer cells.

Nervous system tumors

Cancer in the nervous system present a major health challenge. In children, cancers in the nervous system have the second highest cancer-related mortality, after leukemia (Bray et al., 2018; Ferlay et al., 2019). The most common malignant diagnosis includes neuroblastoma, a tumor presenting in the sympathetic nervous system, and the brain tumor medulloblastoma. Cancer in the nervous system is the 12th most common cause of cancer-related death in the world (Bray et al., 2018; Ferlay et al., 2019) and glioblastoma is the most common malignant brain tumor (Ostrom et al., 2019). The clinical course of brain tumors varies drastically, where patients with glioblastoma (grade IV) have a dismal prognosis of just over a year with the best available treatment, while low grade lesions might be cured with surgery only (Louis et al., 2016). In neuroblastoma, the clinical heterogeneity spans from spontaneous regression of metastatic disease, to high-risk neuroblastoma where only half of the patients are alive 5 years after their diagnosis, despite extensive therapy (Cohn et al., 2009). Nervous system tumors, especially brain tumors, are also a major cause of cancer related morbidity, reduced quality of life, and long-term dependencies on the health care system for survivors (Taphoorn et al., 2010).

New treatments for high-risk neuroblastoma and glioblastoma are highly warranted. In this thesis, we explore different ways of identifying novel targeted treatments for these diagnoses and develop zebrafish xenograft models for treatment evaluation.

Neuroblastoma

Neuroblastoma is the most common extracranial solid tumor in children, with a median age of 1.6 years at diagnosis (London et al., 2005). Neuroblastoma develops in the peripheral nervous system from the developing sympathetic ganglia, originating from the neural crest (Brodeur, 2003; Westerman et al., 2011). The most common primary sites are the adrenal medulla and sympathetic ganglia along the spinal cord, and it spreads primarily to bone marrow, bone, lymph nodes, and liver (DuBois et al., 1999). Some patients with widespread disease limited to skin, liver, and bone marrow still show spontaneous regression and excellent survival, while high-risk patients have poor survival, making neuroblastoma a clinically heterogeneous disease (Cohn et al., 2009). Familial neuroblastoma is often caused by heritable mutations in the receptor tyrosine kinase *ALK* or the transcription factor *PHOX2B* (Carén et al., 2008; Krona et al., 2008; Mosse et al., 2004; Mossé et al., 2008; Trochet et al., 2004).

Risk groups define treatment stratifications

Neuroblastoma patients are treated based on a risk-group system. The risk groups are based on clinical, molecular and histological features of the tumor including tumor spreading (stage), age of the patient, histology (proliferation index and degree of differentiation), amplification of the *MYCN* gene, chromosomal aberrations of 11q, and DNA ploidy (Figure 2) (Ambros et al., 2009; Cohn et al., 2009). Staging is defined as localized, with or without invasion into surrounding structures, or metastatic disease, according to either the International Neuroblastoma Staging System (INSS) or the International Neuroblastoma Risk-Group (INRG) Staging Systems. Neuroblastoma has a separate staging entity, 4S (MS in INRG), for young patients with widespread disease, as these tumors often regress spontaneously and are considered low-risk (Nickerson et al., 2000). Patients older than 1.5 years belong to a higher risk group (London et al., 2005).

The treatment of neuroblastoma varies largely between the risk groups. Low-risk patients have an excellent survival even without treatment, or with either surgery or chemotherapy alone (Strother et al., 2012). For the high-risk patient group, the prognosis is much worse, with only half of the patients sur-

viving after 5 years despite extensive therapy protocols including chemotherapy, surgery, radiation, immunotherapy, stem cell transplantation, and differentiation therapy (Kushner et al., 1994; Matthay et al., 1999; Park et al., 2011; Pinto et al., 2015). During the last decade, large-scale efforts coupling patient clinical and molecular data have started to decipher the molecular characteristics of high-risk neuroblastoma, in an effort to understand more about the mechanisms underlying the disease and to find novel treatments.

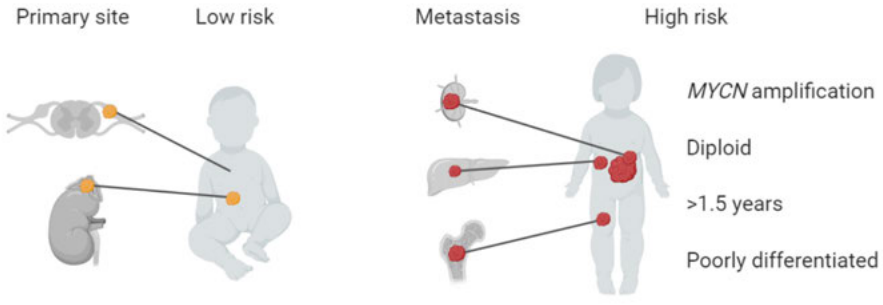

Figure 2. Neuroblastoma primary and metastatic sites. Low- and high-risk patients are stratified based on clinical and molecular markers.

Molecular characterization of neuroblastoma

Childhood tumors typically carry fewer mutations than adult cancers. In neuroblastoma, the average mutation burden is 12 missense somatic mutations, with a higher frequency in high-risk tumors (Molenaar et al., 2012). There are relatively few recurrent mutations between neuroblastoma patients (Figure 3). The most common mutations include activation of the receptor tyrosine kinase ALK and inactivation of the transcription factor ATRX, involved in telomere elongation, affecting approximately 10% of the patients each (Cheung et al., 2012; Molenaar et al., 2012; Pugh et al., 2013). The relative paucity of somatic mutations in targetable proteins makes it difficult to develop targeted therapies for neuroblastoma based on DNA sequencing of the primary tumor alone.

Copy number aberrations occur in many high-risk patients, including gain of the *MYCN* locus on chromosome 2p24 (Schwab et al., 1983), loss of 1p36 (Brodeur et al., 1981; Maris et al., 2000), loss of 11q (Guo et al., 1999), and gain of 17q (Bown et al., 1999; Caron et al., 1996). Chromothripsis (localized chromosomal shredding and subsequent random assembly, leading to structural variations in the genome) have been found to be associated with poor survival, affecting 18% of stage 3 and 4 patients (Molenaar et al., 2012). Structural rearrangements have been associated with alterations in the DNA damage response pathway such as *FANCM* and *FAN1*, which are part of the Fanconi anemia pathway (Molenaar et al., 2012).

MYCN

Amplification of the transcription factor *MYCN*, encoding the N-Myc protein, is the most common genetic alteration in neuroblastoma and is associated with a worse prognosis (Ambros et al., 2009). In normal cells, *MYCN* is expressed during development (Zimmerman et al., 1986). Targeted expression of *MYCN* in tyrosine hydroxylase (*TH*) positive neural crest cells is sufficient for neuroblastoma formation in mice (Weiss et al., 1997). Loss of N-Myc induces cell death and differentiation (Kang et al., 2006). It has been proposed that N-Myc regulates differentiation through miRNA control of nuclear hormone receptors (Ribeiro et al., 2016). Unfortunately, N-Myc is a difficult target for drug development and consequently many studies focus on indirect targeting of N-Myc through reduced *MYCN* transcription, increased degradation of N-Myc by targeting N-Myc phosphorylating proteins, or targeting of downstream effector pathways (Pinto et al., 2015).

ALK

Familial cases of neuroblastoma constitute 1% of the cases and 50% of these cases are associated with activating *ALK* mutations (Devoto et al., 2011; Mossé et al., 2008). In sporadic neuroblastoma, *ALK* is mutated in 8-12% of the patients (Bresler et al., 2014; Carén et al., 2008; Mossé et al., 2008), and relapse patients tend to acquire *ALK* mutations (Schleiermacher et al., 2014). *ALK* mutation is predictive of survival (Pugh et al., 2013). ALK inhibitors are approved for non-small cell lung cancer and targeting of ALK is under clinical investigation, see below.

Telomere maintenance

Recently, replication immortality through telomere maintenance has been proposed to be one of the main risk-determinants in neuroblastoma, along with p53 and RAS pathway alterations (Ackermann et al., 2018). In high-risk tumors, telomere maintenance is obtained through either *MYCN* amplification, *TERT* rearrangements, or *ATRX* mutations, while tumors with spontaneous regression lack these alterations (Ackermann et al., 2018). Further alterations in RAS and/or p53 pathways increase the risk for the patients to very high-risk (Ackermann et al., 2018).

Whole genome sequencing of high and low-risk patients have identified genomic translocation in the 5p15.13 region in 21-23% of the tumors, leading to increased *TERT* expression by placing an active enhancer in proximity to the *TERT* gene (Peifer et al., 2015; Valentijn et al., 2015). Cases with *TERT* rearrangements show increased telomere length (Valentijn et al., 2015) and the alteration is almost mutually exclusive to *MYCN* amplification and *ATRX* mutations (Peifer et al., 2015; Valentijn et al., 2015). However, *MYCN* amplified tumors upregulate *TERT* mRNA expression (Peifer et al., 2015). No patient samples showed *TERT* promoter mutations (Lindner et al., 2015), which

is common in glioblastoma. In high-risk tumors without *TERT* upregulation, alternative lengthening of telomeres (ALT) was activated (Peifer et al., 2015). *TERT* in itself is a difficult drug target, but the region upstream of *TERT* shows Polycomb modification characteristics (Valentijn et al., 2015) and consequently, the *TERT* expression could possibly be regulated by inhibition of the Polycomb repressive complex.

Loss-of-function mutations in *ATRX* are associated with older patients and they appear in all patients older than 12 years (Cheung et al., 2012). Structural variation in the *ATRX* gene leads to decreased expression (Molenaar et al., 2012). N-terminal deletions in the *ATRX* gene activate REST, a transcription factor which repress neuronal differentiation programs and recruit Polycomb Repressive Complex 2 (PRC2), and can possibly be targeted with EZH2 inhibition, a subunit of the PRC2 (Qadeer et al., 2019).

Super-enhancers in neuroblastoma

Due to the low frequency of mutations in neuroblastoma, there has been a recent interest in understanding the neuroblastoma epigenetic regulation. It has long been known that neuroblastoma cells can switch between epithelial-like and neuroblast-like cell states (Ross et al., 1983), indicating an epigenetically regulated phenotype switch. Recent studies have shown that isogenic neuroblastoma cells can adopt either a neural crest cell/mesenchymal-like state (MES/NCC) or an adrenergic/noradrenergic cell state (ADRN; Figure 3), which can be separated based on CD133 expression levels (Boeva et al., 2017; van Groningen et al., 2017). Sorted cell populations can switch phenotype both *in vitro* and *in vivo* (van Groningen et al., 2017). The cell identity is determined by core transcriptional regulatory networks in which a set of transcription factors increase their expression through a feed-forward loop, mainly driven by the activation of super-enhancers, and which are identified by an increased density of histone 3 lysine 27 acetylation and trimethylation (H3K27ac, H3K27me3) epigenetic marks (Boeva et al., 2017; van Groningen et al., 2017). The transcription factors regulating the ADRN identity include GATA3 and PHOX2B, while MES/NCC-cells are driven by AP-1 transcription factors, consisting of heterodimers of FOS and JUN family members (Boeva et al., 2017; van Groningen et al., 2017). NOTCH and PRRX1 expression reprogrammed cells into a more MES/NCC state (van Groningen et al., 2017, 2019). MES/NCC cells were more resistant to chemotherapy, suggesting that targeting of MES/NCC cells or transforming them into ADRN cells might be a possible treatment strategy (Boeva et al., 2017; van Groningen et al., 2017).

Recurrent neuroblastoma

Neuroblastoma patients succumb to refractory or relapsed disease. In relapsed neuroblastoma, the mutational burden increases (Eleveld et al., 2015;

Schramm et al., 2015). Many mutated genes are involved in epithelial to mesenchymal transition (EMT) processes (Schramm et al., 2015) or activation of the RAS-MAPK pathway, including *ALK, KRAS, HRAS, PTPN11*, or *NF1* mutations (Eleveld et al., 2015). Patients also acquire chromosomal aberrations characteristic of aggressive and metastatic disease, such as chromosome 9 deletions harboring the *DOCK8* locus (Schramm et al., 2015), partial loss of chromosome 6, 1p, or 11q, or homozygous deletion of *CDKN2A* (Eleveld et al., 2015). Interestingly, the transcriptional landscape is altered in relapsed neuroblastoma without major changes in the epigenetic profile (Schramm et al., 2015).

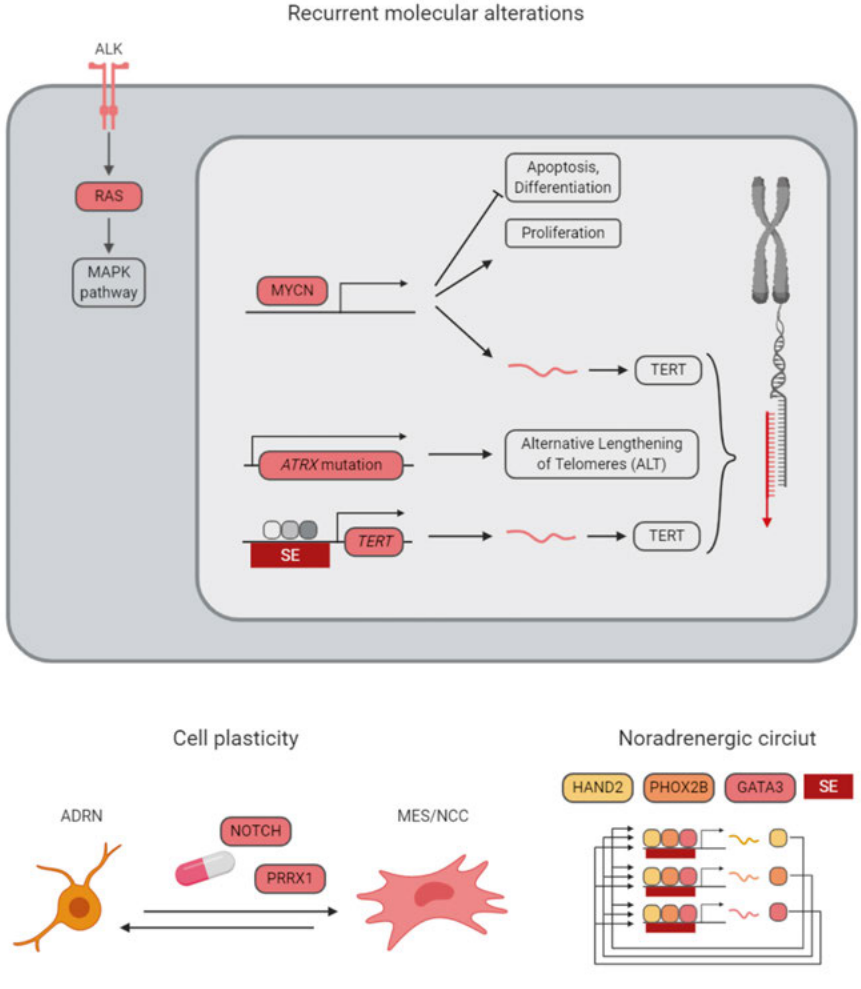

Figure 3. Recurrent genomic alterations and cell plasticity in neuroblastoma. SE: Super Enhancer.

Neuroblastoma evolution and heterogeneity

Despite the higher frequency of mutations in recurrent neuroblastoma, relapsed tumors have a lower heterogeneity of chromosomal aberrations than primary tumors (Schramm et al., 2015). Many mutations in relapse samples are shared with the primary tumor (Eleveld et al., 2015), though the spectrum of mutations differs between locoregional and metastatic relapses. Locoregional relapses show a broader spectrum of acquired mutations than metastatic relapses (Schramm et al., 2015), suggesting that several local subclones could be responsible for the relapse and that tumor evolution occur both temporally and spatially.

Analysis of chromosomal rearrangements has revealed four different evolutionary trajectories in neuroblastoma, with high regional heterogeneity indicating a worse prognosis (Karlsson et al., 2018). Specifically, clonal sweeps, in which one clone takes over and dominates an anatomical region, and mutational explosions were both predictors of recurrence (Karlsson et al., 2018). This motivates the collection of biopsies from several locations and that new biopsies are collected upon recurrence.

Emerging targeted therapies for neuroblastoma

Emerging therapies for neuroblastoma aim to target specific molecular alterations or specifically expressed proteins. In an early phase clinical trial, only 9% of *ALK* mutated patients responded to treatment with the ALK inhibitor crizotinib (Mossé et al., 2013), possibly due to activating mutations in the *ALK* kinase domain (Bresler et al., 2014). Ongoing clinical trials are trying to overrule the crizotinib resistance using second-generation ALK inhibitors such as ASP3026, loratinib, and LDK-378 (Li et al., 2016)(clinicaltrials.gov, NCT03107988, NCT01742286). Recent development of an antibody-drug conjugate targeting ALK has showed promising effects *in vivo*, hopefully treating neuroblastoma cells that would otherwise develop a resistance through mutation of the receptor (Sano et al., 2019). In relapse samples, some of the mutations in RAS-MAPK pathways might be targeted with MEK inhibitors (Eleveld et al., 2015).

MYCN, *TERT*, and *ATRX* and are three commonly altered genes in neuroblastoma. Unfortunately, targeting of the encoded proteins with small molecule inhibitors has proven difficult. Instead, treatment strategies have aimed to indirectly target N-Myc protein levels as it has proven difficult to regulate the protein directly. Current efforts are made to downregulate *MYCN* transcription by the use of BET bromodomain inhibition (Puissant et al., 2013) or protein destabilization through PI3K (Chesler et al., 2006), ROCK (Dyberg et al., 2017), or Aurora A kinase interference (Brockmann et al., 2013; DuBois

et al., 2018). Inhibition of the N-Myc downstream target ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis, using the inhibitor DFMO, is also under clinical evaluation (clinicaltrials.gov: NCT01586260, NCT02030964), though a treatment combination with polyamine uptake might be needed (Gamble et al., 2019).

Other therapies in clinical studies include targeted radiotherapy using the radioactive norepinephrine receptor ligand 131I-MIBG (DuBois et al., 2015)(NCT03126916) and immunotherapy targeting the ganglioside 2 (GD2) antigen using monoclonal antibodies or genetically engineered T-cells expressing chimeric antigen receptors (CAR T-cells) (Louis et al., 2011) (NCT02107963). Clinical evaluation of the use of targeted DNA sequencing of neuroblastoma for precision medicine is ongoing (NCT02868268).

Despite the extensive effort in identifying targeted therapies, little is known about therapeutic targets beyond gene mutations and tissue-specific gene expression. Given the low frequency of mutations in neuroblastoma, researchers have screened for epigenetic regulators that affect neuroblastoma growth and differentiation (Lochmann et al., 2018; Veschi et al., 2017). A recent epigenetically focused drug screen aiming to induce differentiation found that an H3K27 demethylase inhibitor induced differentiation in neuroblastoma, including retinoic-acid resistant cell lines (Lochmann et al., 2018). Another combined siRNA and chemical screen on epigenetic regulators identified SETD8, a histone 4 lysine 20 methyl (H4K20me1) transferase, as a possible target in neuroblastoma (Veschi et al., 2017). SETD8 downregulation or inhibition reactivated p53 pro-apoptotic functions and induced differentiation (Veschi et al., 2017). Considering the cell plasticity and epigenetic regulation in neuroblastoma, new ways of identifying targeted therapies based on cell state and transcriptional programs can hopefully increase the repertoire of possible treatments for the high-risk disease.

Glioblastoma

The most common malignant brain tumor is glioblastoma, accounting for almost half of all primary malignant brain tumors and 15% of all brain tumors (Ostrom et al., 2019). Glioblastoma is more or less incurable, with a 6.8% 5-year survival rate (Ostrom et al., 2019) and a median survival time of 15 months with standard-of-care therapy (Stupp et al., 2005). In the western population, more than 3 of 100.000 individuals are diagnosed with glioblastoma every year, with a higher incidence in the Caucasian population (Ostrom et al., 2019). The median age of diagnosis is 65 years, the incidence increasing with age, and few patients are diagnosed before age 40 (Ostrom et al., 2019). Age of the patient is highly correlated with disease outcome and younger patients have a better prognosis (Louis et al., 2016). Symptoms of glioblastoma are commonly unilateral and are related to the site of the tumor or increased intracranial pressure and can be manifested as seizures, nausea, neurocognitive changes, a reduction in mobility, or severe headaches (DeAngelis, 2001).

Glioblastoma is a highly invasive, but rarely metastatic, grade IV astrocytoma (Louis et al., 2016). Histological features of glioblastoma include high cellularity, poor differentiation, high mitotic activity, nuclear atypia, heterogeneity in cellular morphology, extensive local and distant infiltration, microvascular proliferation, and necrosis (Louis et al., 2016). Glioblastoma is divided into isocitrate dehydrogenase (IDH) 1/2-wild type (primary) glioblastoma, accounting for 90% of glioblastoma, and IDH-mutant (secondary) glioblastoma, which progresses from lower stage brain tumors (Ceccarelli et al., 2016; Nobusawa et al., 2009; Ohgaki and Kleihues, 2013). Secondary glioblastoma has a slightly better survival prognosis than primary glioblastoma, suggesting a different biology between the two entities (Parsons et al., 2008). The IDH-mutant subgroup is also associated with methylated CpG islands in the DNA, a phenotype called G-CIMP (Ceccarelli et al., 2016; Noushmehr et al., 2010).

A glioblastoma tumor can occupy a large part of a cerebral lobe already at presentation, even though the duration of symptoms has been very short. The most common sites are the subcortical white matter and deep grey matter in the temporal lobe, parietal lobe, frontal lobe, and occipital lobe (Lai et al., 2011). Infiltration into the cortex, along the corpus callosum and into the contralateral hemisphere is common (Louis et al., 2016). For IDH-mutant glioblastoma, the localization is predominantly the region surrounding the rostral lateral ventricles (Lai et al., 2011), as compared to the more widespread origin

of IDH-wild type glioblastoma. Radiologically, the tumor is visible as an irregular, contrast enhancing mass with a dark necrotic center. The infiltration into surrounding tissues is very rapid and cells can spread along white matter tracts, or form Scherer structures (Scherer, 1938), including perineuronal satellitosis, perivascular aggregation and subpial spread. The mechanism behind glioblastoma infiltration is not completely understood, but involves activation of cell motility programs, cell-cell and cell-matrix interactions (e.g. through integrins), remodeling of extracellular matrix (e.g. secretion of proteolytic enzymes such as matrix metalloproteinases), as well as cues from the microenvironment (Bellail et al., 2004; Demuth and Berens, 2004). Despite extensive infiltration, glioblastoma does not commonly spread to other organs, although circulating tumor cells have been found in patient blood, suggesting an immune-surveillance mechanism (Sullivan et al., 2014). CD8+ T-cells might be more prominent in long term survivors (Yang et al., 2010).

Although the cell-of-origin is still under investigation, studies suggest that glioblastoma arise from oligodendrocyte precursor cells (Liu et al., 2011) or neural stem/progenitor cells (Alcantara Llaguno et al., 2009; Lee et al., 2018a). The cell-of-origin affects both tumorigenicity and drug sensitivity, with transformed neural stem cells having a higher tumorigenic potential (Jiang et al., 2017).

Current treatment of glioblastoma

The standard-of-care therapy for glioblastoma is surgery, radiation, and chemotherapy, using the DNA-alkylating agent temozolomide (Stupp et al., 2005). Temozolomide sensitivity is affected by promoter methylation of the DNA repair gene O-6-methylguanine-DNA methyltransferase (MGMT), causing gene silencing (Hegi et al., 2004). Inactivation of the gene leads to a decrease in DNA repair and an increased sensitivity to temozolomide, making MGMT promoter methylation a biomarker of increased temozolomide sensitivity in patients (Stupp et al., 2005). However, treatment with alkylating agents, such as temozolomide, leads to an increased mutation rate due to the lack of functional DNA repair mechanisms, and recurrent tumors commonly show a hypermutation phenotype (Cancer Genome Atlas Research Network, 2008). While the separation of IDH-wild type and -mutant glioblastoma is useful when evaluating patient prognosis, this molecular separation of glioblastoma subgroups has not yet resulted in any molecular-guided treatment options for these different subgroups.

Molecular characteristics of glioblastoma

Compared to neuroblastoma, glioblastoma has a higher mutational burden and more frequently altered pathways. Recurrent genetic aberrations in glioblastoma activate receptor tyrosine kinases (mainly *EGFR*, *PDGFRA*, *MET*, and *FGFR*) and downstream signaling pathways (RAS-BRAF and PI3K pathways), or inhibit their negative regulators (*NF1* and *PTEN*, respectively; Figure 4) (Brennan et al., 2013; Cancer Genome Atlas Research Network, 2008). Glioblastoma cells typically inactivate genes involved in p53 regulation (most common alterations occur in *TP53*, *MDM2*, and *MDM4*), and activate cell cycle regulators upstream of *RB1* (e.g. *CDK4*, *CDK6*, cyclins, and *CDKN2A/B/C*) (Brennan et al., 2013; Cancer Genome Atlas Research Network, 2008) (Figure 4). Increased EGFR signaling can be gained through mutation, overexpression, or deletion of exons 2-7 in the extracellular domain (*EGFRvIII*) leading to constitutively active receptor signaling (Brennan et al., 2013; Huang et al., 1997). *EGFR*-amplified glioblastoma cells are commonly located in the tumor border, suggesting a role for EGFR in promoting invasion (Snuderl et al., 2011). Glioblastoma cells acquire *TERT* promoter mutation or mutations in *ATRX,* involved in telomere maintenance (Ceccarelli et al., 2016; Eckel-Passow et al., 2015). These mutations are mutually exclusive (Ceccarelli et al., 2016). The most common chromosomal aberrations in glioblastoma include gain of chromosome 7, harboring the *EGFR, MET,* and *CDK6* locus (Brennan et al., 2013).

Glioblastoma subgroups and heterogeneity

Glioblastoma is highly heterogeneous, both between patients and within the same patient. In the last decade, extensive efforts have been focused on subgrouping different patients based on their molecular features, aiming to understand the underlying mechanism of this heterogeneity and to adapt treatments based on different molecular phenotypes. In *IDH*-wild type glioblastoma, transcriptional and methylation analysis first suggested four molecular subgroups (Brennan et al., 2013; Sturm et al., 2012; Verhaak et al., 2010), while later studies have confirmed three of these (Ceccarelli et al., 2016; Wang et al., 2017). Named after cellular resemblance, the subgroups are called proneural, mesenchymal, and classical (Verhaak et al., 2010; Wang et al., 2017). The classical subgroup included well-established glioblastoma genomic alterations, such as *EGFR* oncogene amplification or overexpression, loss of the *PTEN* locus on chromosome 10, downregulation of the *CDKN2A/RB1* tumor suppressor pathways, wild type *TP53*, and an upregulation of neural precursor markers (Verhaak et al., 2010). Cells in the proneural subgroup are often *TP53* mutated, have an inactivated *CDKN2A* pathway, an activation of *PDGFRA*

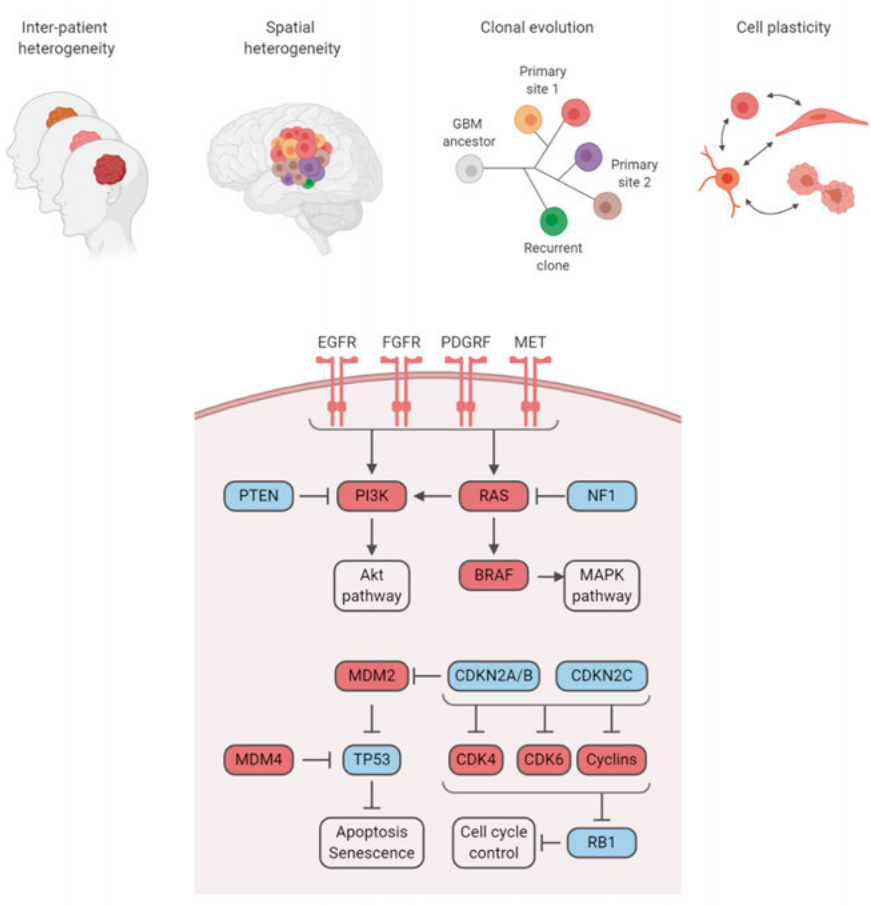

Figure 4. Glioblastoma heterogeneity and molecular characteristics.

through amplification or elevated expression, and express high levels of markers from the oligodendrocytic and neural developmental lineage (Verhaak et al., 2010). Characterization using patient methylation data further divided the proneural subgroup into two distinct groups, where one showed a hypermethylated phenotype (G-CIMP) with a better prognosis, tightly linked to *IDH1* mutation status (Noushmehr et al., 2010). The mesenchymal group has an inactivation of *NF1* and *PTEN* tumor suppressor genes and activation of *MET*, together with upregulation of markers from the astrocyte lineage (Verhaak et al., 2010). These subgroups showed different response to treatments, with more intensive therapy being beneficial for patients in the classical and mesenchymal subgroups, but not in the proneural subgroup (Verhaak et al., 2010).

Further studies have described a more complex model of glioblastoma heterogeneity. Sampling multiple regions of a tumor revealed a mix of subgroups within the same patient (Lee et al., 2017; Sottoriva et al., 2013) and with different driver mutations represented in different spatial regions (Kumar et al., 2014; Snuderl et al., 2011), in line with glioblastoma being a tumor type with

great intratumor (within patient) heterogeneity. Phylogenetic trees describing the evolution of intratumor clones identified sequential copy number alterations, with *EGFR* and *CDKN2A/B* being early hits, followed by *PDGFRA* and *PTEN* alterations (Sottoriva et al., 2013). Analysis of single cell RNAseq and chromosomal aberrations have further confirmed that multiple subgroups are present within the same patient (Patel et al., 2014; Wang et al., 2017). Drug screening on patient samples derived from different regions of the same tumor showed a heterogeneous drug response (Lee et al., 2017). Recent studies of single cells have found that glioblastoma subgroups might rather be different cell states and that glioblastoma cells can exist in four independent cell states (Neftel et al., 2019). Cells from a specific state can repopulate the other states (Neftel et al., 2019), indicating a level of cell plasticity. The state frequency is influenced by copy number alterations or mutations in key glioblastoma genes (*CDK4, EGFR, PDGFRA,* and *NF1*), each promoting one of the four states (Neftel et al., 2019). Glioblastoma heterogeneity and cell plasticity present a major treatment challenge for glioblastoma.

Challenges for glioblastoma treatment

There are several challenges associated with glioblastoma treatment. First, the infiltrative nature of glioblastoma cells results in residual tumor cells after surgery and radiation. Second, the blood-brain barrier and the high intratumoral pressure is a challenge for drug delivery. Third, intratumoral heterogeneity, tumor cell plasticity, and genomic instability promotes the outgrowth of resistant clones (Louis et al., 2016).

The extensive invasiveness of glioblastoma, where cells can spread several centimeters from the originating tumor mass, is a likely cause of tumor recurrence as not all cells will be resectable by surgery. Invading cells will receive lower doses of radiation and are protected from chemotherapy behind a stable blood-brain-barrier, making it difficult for many compounds to access the glioblastoma cells (Giese et al., 2003). The infiltrating cells also show a reduced proliferation rate (Darmanis et al., 2017), indicating that these cells might be less sensitive to conventional therapy targeting cell proliferation. Single cell RNAseq of patient material has suggested common mechanisms for tumor infiltration, as infiltrating cells from different patients show similar transcriptional profiles (Darmanis et al., 2017). Motility might be induced through activation of PI3K pathways, as multifocal tumors have a higher frequency of *PIK3CA* mutations than solitary tumors and are more sensitive to PI3K/AKT/mTOR inhibitors (Lee et al., 2017).

The heterogeneity within the tumor constitutes a major challenge for the treatment of glioblastoma. At recurrence, 63% of glioblastomas switch the transcriptional subtype (Wang et al., 2016), adopting a more mesenchymal phenotype (Wang et al., 2017). Some tumors become hypermutated due to

the temozolomide treatment (Wang et al., 2016). Studies on clonal rates and primary-recurrence similarities have shown that clones responsible for patient relapse exist years before diagnosis (Wang et al., 2016). Only a subset of mutations are shared between the primary and recurrent tumor mutations (Kim et al., 2015a, 2015b; Wang et al., 2016), suggesting that targeting of early genetic events, which are shared between a larger proportion of the tumor cell population, has been more promising than targeting of subclonal events (Lee et al., 2017). Interestingly, shared mutations are typically not glioblastoma-associated driver genes (Wang et al., 2016) and early events might not be necessary for tumor maintenance, only for tumor initiation, which limits the number of suitable drug targets (Lee et al., 2017).

Genomic heterogeneity is coupled to the functional heterogeneity of tumor cells (Meyer et al., 2015). While some patient subclones are sensitive to treatment, other subclones present with a multi-therapy resistance phenotype. This is a plastic process in the cells as single glioblastoma cells can be expanded to include both therapy sensitive and resistant subpopulations (Segerman et al., 2016). Multi-therapy resistance is shifted along a proneural-mesenchymal axis, with mesenchymal cells being more resistant to treatment (Segerman et al., 2016). The plasticity of glioblastoma cells makes it difficult to identify new treatments for glioblastoma patients.

Despite extensive efforts in glioblastoma characterization, targeting of major driver events, such as EGFR, have not resulted in any clinical benefit (Lee et al., 2015; Thiessen et al., 2010; Uhm et al., 2011; Weller et al., 2017). The resistance is possibly due to dynamic clonal populations of cells harboring *EGFR* mutant extrachromosomal DNA (Nathanson et al., 2014) or through alternative mechanisms such as *NGR1* or PI3K/AKT/mTOR pathway upregulation (Lee et al., 2018b). Most likely, several events need to be targeted simultaneously to hit different mechanisms of glioblastoma growth and invasion. To identify novel therapies for glioblastoma, we need reliable models that capture the whole spectrum of heterogeneity and plasticity of glioblastoma cells and new methods for stratifying patients based on drug sensitivity.

Strategies to identify targeted therapies

Finding new specific drug targets for high-risk neuroblastoma and glioblastoma remains a matter of crucial importance. During the last decade, extensive analysis of patient genomic data has revealed druggable molecular alterations in several tumor types, some which have shown *in vivo* relevance and been approved for clinical use, including *HER2* overexpressing breast cancer (Ryan et al., 2008; Slamon et al., 2001) and *BRAF V600E* mutated melanomas (Chapman et al., 2011). Targeting of specific alterations has therefore become a tractable option for cancer drug discovery.

However, inhibition of single targets based exclusively on patient mutation data is not always fruitful. For example, glioblastoma patients with EGFR alterations do not respond to *EGFR* inhibition (Lee et al., 2015; Thiessen et al., 2010; Uhm et al., 2011; Weller et al., 2017). With relatively few recurrent mutations in neuroblastoma, there are limited possibilities for targeted therapies based on mutations and DNA sequencing alone.

Extensive data collection from functional screens in serum-cultured cell lines and patient-derived cells, using drugs, siRNA, shRNA, or CRISPR technologies, have found that drug sensitivity does not necessarily correlate with mutated single targets, but rather with molecular profiles containing many types of data (Twomey et al., 2017). Future treatments will likely rely on (i) robust patient stratifications using different data types to identify the patients that will benefit from a treatment, (ii) innovative data integration methods that find novel drug targets beyond recurrent mutations, and (iii) drug combinations to overcome resistance mechanisms. Here, I describe different strategies for drug target identification.

Mapping genomic alterations in patient tumors

The massive amount of available cancer data has led to new opportunities in cancer drug discovery. Patient clinical and molecular data, typically copy number variation (CNV), transcriptome, and exome collections, is at the center of drug discovery. During the last decade, an increasing amount of molecular and clinical data from cancer patients has been collected and is available in public data sources. The Cancer Genome Atlas (TCGA) has characterized the molecular landscape in more than 11 000 patients covering 33 cancer diagnosis, including glioblastoma (Bailey et al., 2018; Brennan et al., 2013;

Ding et al., 2018; Liu et al., 2018; Sanchez-Vega et al., 2018). Neuroblastoma patients have been extensively characterized in several studies (Cheung et al., 2012; Molenaar et al., 2012; Pugh et al., 2013; Zhang et al., 2015). Additionally, more than half of the tumors, both adult and pediatric, harbor potentially druggable events (Bailey et al., 2018; Gröbner et al., 2018). The landscape of cancer mutations from almost 1.4 million tumor samples have been summarized in the Catalogue Of Somatic Mutations In Cancer (COSMIC) resource, including information of mutation druggability and impact on protein structure and function (Tate et al., 2019). Understanding common mechanisms within each disease and differences between cancer diagnoses have helped to identify several links between genomic variation, such as oncogene activation, and patient outcome. Recent data collection extends to include pan-cancer enhancer expression (Chen et al., 2018), metabolome (Li et al., 2019; Peng et al., 2018), immunoprofiling (Thorsson et al., 2018), influence of tumor cell-of-origin (Hoadley et al., 2018), splicing variations (Jayasinghe et al., 2018; Seiler et al., 2018), and specific pathway alterations, such as MYC (Schaub et al., 2018). In coming years, large-scale and pan-cancer collections of proteome, single cell, and spatial influence on patient tumors is expected to emerge. Together, these data give a solid understanding of cancer genome variations and map out possible mechanisms for targeted interventions. However, as previously discussed, not all alterations are possible to target, nor might they be crucial for tumor maintenance or cause relapse. This has led to the need to define cancer molecular dependencies.

Mapping cancer cell line vulnerabilities

The sensitivity of cancer cells to drugs or gene interference has been extensively studied in established cell lines covering multiple cancer diagnosis, using large-scale screening methods. Functional screening can be done using several technologies, including CRISPR (gene knockout), siRNA/shRNA (gene expression knockdown), or drug (typically targeting proteins). Readout should be high-throughput and reflect responses of interest, e.g. reduced cell viability (measured through metabolic activity with AlamarBlue or MTT), reduced proliferation (EdU), altered cell cycle distribution (DNA content analysis in combination with EdU/BrdU or FUCCI), or increased apoptosis (e.g. cleaved caspase 3/7). Linking cell line molecular data to drug sensitivity is the gold standard for identifying biomarkers that can predict cancer vulnerabilities. Databases containing cancer cell line sensitivity to chemical compounds (Barretina et al., 2012; Garnett et al., 2012; Rees et al., 2016; Yu et al., 2016) and gene interference (Cowley et al., 2014; Gönen et al., 2017) have been linked to cancer cell line molecular data (Ghandi et al., 2019), providing a cancer Dependency Map (Depmap) database (Tsherniak et al., 2017) of asso-

ciations between cell type, molecular status, and vulnerabilities. These resources are valuable tools for cancer drug target and biomarker discovery, but do not provide information about the mechanisms behind target vulnerability, which is necessary for understanding drug resistance mechanisms and how drugs affect cellular pathways involved in cancer maintenance. Another drawback in these large efforts is the choice of cell models, as established cell lines, which have been cultured in serum over decades, tend to drift away genetically from the original patient tumor and do not resemble the patient tumors as well as primary cell cultures (see next chapter). Functional screens in primary cell cultures will be a necessary extension to screens in traditional cells lines to fully understand the diversity of responses within a disease entity.

Mapping transcriptional effects of drugs

The need for systematic mapping of molecular responses in cells after external intervention led to the early development of the ConnectivityMap database (Lamb et al., 2006), containing transcriptional response profiles in a set of cancer cell lines, and have successfully been used to identify new targets in neuroblastoma (Preter et al., 2009). More recent developments of the pipeline have resulted in the vastly extended L1000/LINCS dataset, with 1.3 million transcription response profiles after shRNA, drug, and overexpression constructs (Subramanian et al., 2017). Drugs mimicking or reversing a specific signature are searchable using the characteristic direction signature search engine (L1000CDS2) (Duan et al., 2016). With improved data processing, e.g. by the Remove Unwanted Variation (RUV) method, it is possible to remove noise in the L1000/LINCS data, improving its potential for discovery (Lönnstedt and Nelander, 2017). Continued mapping of patient and cell line responses to drugs will be of high importance for discovery and mechanism of novel targets in cancer. Ongoing effects in the LINCS project aim to understand the drug effect on the proteome. While this type of data might reveal more important drug mechanisms, it is not yet developed to the same extent as transcriptome analysis, thus, most studies are still focused around transcriptional analysis.

Data integration reveals possible targets and mechanisms

Interpretation of large-scale data is becoming one of the central challenges of biomedical research. By combining large-scale databases using integrative analysis, connections between patient data, cell vulnerabilities to drugs, and molecular mechanisms can be understood (Figure 5). For example, analyzing

L1000 signatures with gene set enrichment analysis (GSEA) (Subramanian et al., 2005) can reveal novel functions of drugs or identify altered pathways. Other databases for data interpretation contain information on drug-protein interactions (STITCH) (Szklarczyk et al., 2016), protein-protein interactions (STRING) (Szklarczyk et al., 2015), and protein-target specificity (Chemical Probes Portal) (Blagg and Workman, 2017) and can also be used to identify drug target protein networks for a specific cancer. An increasing number of tools are designed to help biomedical researchers integrate and obtain an overview of these different levels of data. For instance, visualization of connections within large-scale data, e.g. through a network of drug-mutation correlations or drug-target interactions, help researchers to interpret data relations. Tools for this include Cytoscape (Shannon et al., 2003) or web applications such as STITCH or STRING. As data collections increase exponentially, the development of novel tools for data integration and interpretation will become invaluable for our understanding of biology and identify novel targets.

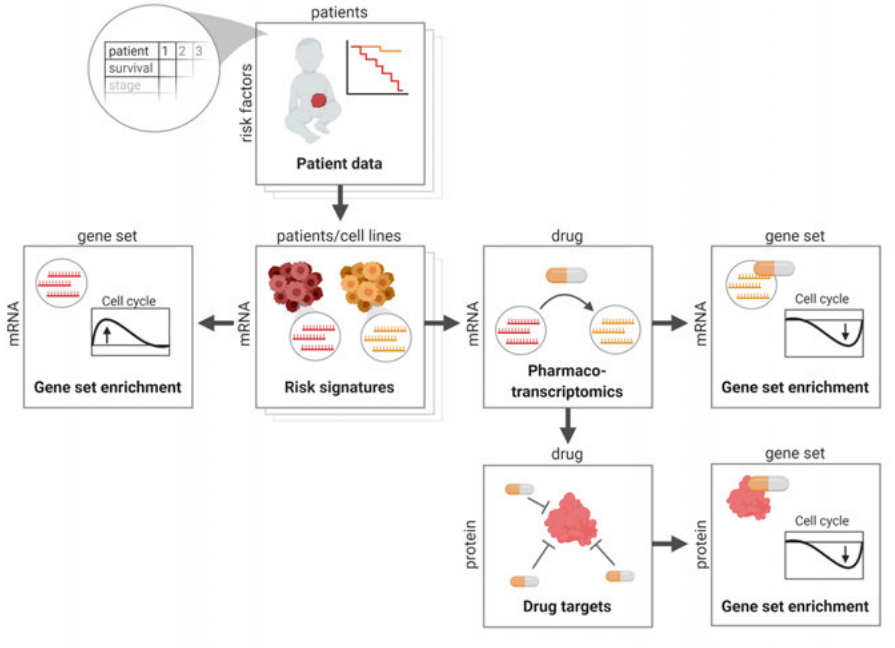

Figure 5. Integration of data resources. Aligning and integration of data matrices covering different aspects of cancer biology opens up new opportunities for drug target discovery.

Mapping synergistic drug pairs

Although targeted therapy has shown promising initial results in clinical studies, many patients suffer from relapse due to low *in vivo* efficacy of drugs or

acquired resistance (Infarinato et al., 2016; Neel and Bivona, 2017). One way of overcoming this challenge is to combine drugs for potentiating effects or to avoid resistance mechanisms. The power of combination therapies has previously been shown for other diseases, such as beta-lactam antibiotics and beta-lactamase inhibitors in bacteria with acquired beta-lactam resistance (Essack, 2001). Other benefits of drug combinations are that dual targeting of a crucial target or pathway is more difficult for a biological system to compensate for, and that lower doses of drugs can achieve the same effect, potentially leading to decreased severity of side effects (Zimmermann et al., 2007). Analysis of drug interactions is mainly based on the idea that drug pairs can be either *antagonistic* and inhibiting the effect of each other, *additive*, with the drug combination showing similar effects as would be expected from the two drugs alone, or *synergistic*, showing a stronger effect together than predicted from the single drug effects. Synergistic drug combinations could be the effect of (i) synthetic lethality, where compensatory pathways are targeted together, (ii) dual targeting of subpopulations of cells with mutually exclusive mechanisms for cell growth and survival, or (iii) targeting of resistant phenotypes showing up as a result of one of the treatments. There are examples of *in vitro* synergistic drug combinations showing promising results in early clinical trials (Finn et al., 2015), making synergistic drug pairs a tractable strategy for treatment development. Identifying synergistic interactions will also help us understand cancer biology and reveal possible treatment combinations.

Experimental models of neural cancers

To evaluate the potential novel drug candidates for neuroblastoma and glioblastoma, *in vitro, in vivo* or *ex vivo* models are used. The models aim to replicate tumor phenotype and molecular responses and are used for mechanistic studies on cellular pathways, such as oncogene inhibition, migration/invasion, tumor differentiation, tumor initiation capacity, or apoptosis. No model perfectly mirrors what happens in patient tumors, but different models can be used to study a panel of functions and mechanisms. Given the large heterogeneity within glioblastoma, studies on glioblastoma cells need to cover a large spectrum of glioblastoma phenotypes and genotypes, for downstream patient stratifications and personalized treatments. Below, I discuss different models for preclinical drug evaluation.

Cell based models of neural cancers

Established cell lines, passaged for many decades in serum-containing media, have a long tradition as tumor models. However, these cell lines drift away genetically from the original tumor as they evolve rapidly in culture (Ben-David et al., 2018; Lee et al., 2018b). Cell cultures derived from patients with neuroblastoma or glioblastoma and that are cultured under defined serum-free conditions more closely resemble the patient tumor (Lee et al., 2006; Persson et al., 2017; Pollard et al., 2009). These culturing conditions enrich for cancer stem cells, a population of cells that can give rise to new tumors and are thought to be intrinsically more resistant to therapy. Thus, patient-derived cell cultures should be used for state-of-the-art drug evaluation. As patients show both inter and intratumor heterogeneity, cell models covering the phenotypic spectrum, e.g. different subgroups of glioblastoma, are very valuable. Patient-derived cell cultures might also have implications in precision medicine in the clinic when used to predict patient treatment response, an approach which is now in clinical trials (Lee et al., 2018b) (NCT03997617).

In vivo models of neural cancers

The drug development process relies on *in vivo* validation of novel treatments in different animal species before entering into humans. Rodent and zebrafish

models of glioblastoma and neuroblastoma include xeno- or allograft transplantation of cancer cells and transgenic models that spontaneously develop disease, either through germline, somatic conditional, or virus-introduced gene alterations. For example, targeted overexpression of *MYCN* in migrating neural crest cells induces neuroblastoma (Weiss et al., 1997) and overexpression of Ras and Akt in neural progenitors or viral introduction of *PDGFB* induces glioblastoma formation (Hambardzumyan et al., 2009; Holland et al., 2000; Uhrbom et al., 1998). While genetic models have many advantages, including a retained immune system, these models develop slowly and sometimes with low penetrance or variable latency, making such models unsuitable for high-throughput drug evaluation. Tumors established by orthotopic transplantation of neuroblastoma or glioblastoma cells directly from patient material (patient-derived xenografts) closely mimic the original tumor but require immune deficient animals for successful transplantation and also show a slow progression (Braekeveldt et al., 2015; Wang et al., 2009).

Rodent models have been extensively used for drug evaluation, but also have many limitations. First, the cost for housing and handling the mice or rats limits the scope of a study. Secondly, the progression of malignant phenotypes, such as cell migration, differentiation, cell plasticity, or interaction with the microenvironment, cannot be followed in mouse studies, as *in vivo* imaging is limited to luciferase reporter systems of tumor growth. This has led to the need for novel cancer models where treatment effects on different phenotypes can be evaluated.

Using zebrafish for drug evaluation

The zebrafish (Danio rerio) has become a useful model organism for studying cancer phenotypes and the effect of treatments. The first studies evaluating novel targets based on zebrafish screens have now made it into the clinical trials, and co-clinical trials evaluating zebrafish xenografts as a predictor of patient drug sensitivity are under evaluation (NCT03668418, NCT03999684) (Mandelbaum et al., 2018). Zebrafish have acquired recent attention in cancer drug evaluation and have many advantages compared to rodent models. By injecting CRISPR gene editing constructs into the fertilized egg, genetic models are relatively easy to generate compared to rodent counterparts. In addition to the reduced cost and ease of maintenance, the life cycle is considerably shorter and several hundred offspring can be obtained weekly, providing a high-throughput model. Several studies have used engrafted zebrafish embryos, which lack an active immune system, for drug screening on GFP-tagged cancer cells. Genetically modified zebrafish strains that lack pigmentation, so called *casper* fish (White et al., 2008), have further increased the optical clarity of the embryos, making it possible to follow drug effect on can-

cer cells at a single cell resolution. Other genetically modified zebrafish models visualizing the vasculature (flk:EGFP, fli1:EGFP) and macrophages/microglia (mpeg1:mCherry, mpeg1:EGFP), have been used to understand the relationship between the tumor cells and the microenvironment, as well as the blood-brain barrier penetration potential of anti-glioblastoma compounds (Hamilton et al., 2016; Pudelko et al., 2018; Zeng et al., 2017).

A concern regarding zebrafish xenografts is the mismatch between zebrafish and human cells in optimal culture temperature. Zebrafish are housed in 28 °C and the embryo survival is drastically decreased over 35 °C, while mammalian cells grow optimally at 37 °C. Multiple studies have agreed that a temperature of 30-35 °C is a reasonable tradeoff between cell and zebrafish temperature sensitivity (Geiger et al., 2008; Pudelko et al., 2018; Yang et al., 2013a). Recently, a new immunodeficient zebrafish strain has shown higher temperature tolerance and is suitable for xenotransplantation in adult fish (Yan et al., 2019).

For glioblastoma, several zebrafish models exist. Transgenic lines expressing *KRAS*(G12V) in neural progenitor or stem cells spontaneously develop brain tumors (Ju et al., 2015). However, the tumor progression rate and penetrance is low, with 40% of the fish harboring a tumor after 6 months, making it a less tractable model for drug evaluation. Zebrafish xenograft models of glioblastoma utilizes fluorescently tagged established cell lines, such as U87MG and U251MG, where tumor cells are injected into the egg yolk of 4-48 hour post fertilization (hpf) embryos, to study the effect on tumor migration, influence of microenvironment, and effect of radiation and temozolomide treatment (Geiger et al., 2008; Yang et al., 2013b, 2013a). Other studies have refined the methodology by injecting established glioblastoma cell lines orthotopically into the brains of zebrafish embryos to study the interaction with microglia and evaluate the effect of therapeutics (Hamilton et al., 2016; Wehmas et al., 2016). Little is known about the growth of patient-derived cell lines, but one study suggests a heterogeneous growth, reflecting the heterogeneity of glioblastoma in humans (Welker et al., 2016).

Transgenic zebrafish models of neuroblastoma include *MYCN* overexpression under the control of the dopamine β hydroxylase (dβh) promoter. Overexpression of *MYCN* alone show a low tumor penetrance of only 20%, as the induced hyperplasia in sympathoadrenal neuroblasts of the zebrafish interrenal gland starts to spontaneously regress at 5.5 weeks in many of the fish (Zhu et al., 2012). To improve penetrance, *MYCN* models of neuroblastoma include *ALK* or *LMO1* co-expression (Zhu et al., 2012, 2017). However, these models still have a slow tumor progression, with 50% penetrance after >10 weeks, making it unsuitable for high throughput screenings. Published xenograft models of neuroblastoma are limited to transplantation of SK-N-BE(2) to the egg yolk and have been used to assess the combination effect of ALK

and HDAC8 inhibitors (Shen et al., 2018). Together, zebrafish models of glioblastoma and neuroblastoma show promise for future cancer biology and drug discovery studies, however, patient-derived models are still lacking.

Present investigations

Given the complex nature of glioblastoma and neuroblastoma, drug target identification relies on robust analysis using different strategies and must be tailored for each cancer. In this thesis, we explore three different ways of identifying novel drug targets and develop zebrafish models of neuroblastoma and glioblastoma using patient-derived cell cultures.

Paper I
Integrative discovery of treatments for high-risk neuroblastoma

In paper I, we identified novel drug targets for high-risk neuroblastoma, which is a relatively rare cancer with few recurrent and druggable mutations, as previously mentioned. Using a data integration strategy, we combined large-scale clinical-transcriptome data from patients (Molenaar et al., 2012; Pugh et al., 2013; Zhang et al., 2015) with transcriptome-drug profiling databases (Subramanian et al., 2017) and drug-target interaction networks (Szklarczyk et al., 2016), identifying links between patient risk and drug targets. Using this strategy, we found more than 80 drug targets that are predicted to have an effect on high-risk neuroblastoma. Screening 12 predicted compounds on cultured cell lines from patient-derived xenografts, we found that transcriptional changes could be predicted with high accuracy. Predicted drugs inhibit high-risk associated features. The most promising target was cannabinoid receptor 2 (CNR2), where target activation reduced N-Myc protein levels and induced differentiation and apoptosis. Tumor growth was inhibited in a novel zebrafish model of neuroblastoma developed for this study, which could be confirmed in an established mouse xenograft model. In summary, paper I describes an innovative way of identifying novel targeted therapies for a rare cancer disease using data integration, with predicted targets having an *in vivo* effect in two animal models of neuroblastoma. The new algorithm, TargetTranslator, is provided as a web tool on targettranslator.org.

Paper II

A drug association map of glioblastoma informs precision targeting of p53-dependent metabolic states

In paper II, we screened more than 100 glioblastoma patient-derived cell lines for sensitivity to over 1500 drugs to find novel targets and subgroups of patients that respond to specific drug interventions. First, in extension to the human glioblastoma cell culture collection (HGCC) (Xie et al., 2015), we expanded cell cultures from glioblastoma patients. Molecular characterization of the cell lines (expression, mutation, methylation, copy number alteration) showed that cell lines cover all the molecular subgroups and contain common alteration in glioblastoma patients (TCGA). Second, cell lines were used in an extensive three phase drug screening effort and drug sensitivity was linked to the cell line molecular profile. One interesting novel target with anti-glioblastoma activity was the sigma receptor, which was linked to glioblastoma lipid metabolism and could be synergistically potentiated by HMGCA inhibition, the enzyme responsible for the rate limiting step of cholesterol synthesis. However, most strikingly was a two-way separation of proteasome inhibitor sensitive and resistant cell lines. Proteasome inhibitor sensitivity was robust between screens and could be predicted in a separate cohort of patient-derived cell lines. Mechanistically, the proteasome inhibitor sensitive cell lines had a low activation of p53 response genes, notably genes acting as redox regulators. Treating cells with antioxidants reduced the sensitivity, linking proteasome sensitivity to cellular redox potential. This study has future implications in precision medicine for proteasome inhibitors and adds sigma receptors to the list of potential anti-glioblastoma therapies.

Paper III

ZBTB16 orchestrates growth and invasion in glioblastoma

In paper III, we identified ZBTB16 as a glioblastoma oncogene in an siRNA screen targeting 1112 gene transcripts in 11 patient-derived cell cultures from the HGCC consortium. Knockdown of *ZBTB16* reduced tumor cell proliferation and invasion *in vitro*, while overexpression increased the growth rate. Gene set enrichment analysis of differentially expressed genes in four cell lines found that genes involved in cell cycle progression - E2F targets (active under G1/S), mitotic spindle, and G2M - were downregulated upon *ZBTB16* knockdown. A role for ZBTB16 in cell cycle regulation was supported by the reduction of EdU staining, a proxy for DNA synthesis during S-phase, in cells with reduced levels of *ZBTB16*. We further found that *ZBTB16* knockdown

reduced Cyclin B1 and Myc protein levels, and that Myc targets were negatively enriched in *ZBTB16* knockdown cells. In a novel zebrafish xenograft model of glioblastoma, overexpression of *ZBTB16* increased invasion of tumor cells into the developing brain. Together, these data indicate a role of *ZBTB16* as an oncogene in glioblastoma, regulating cell proliferation and invasion.

Paper IV

Real-time evaluation of glioblastoma treatments in patient-specific zebrafish xenografts

In paper IV, we developed a high-throughput method for *in vivo* validation of novel drug targets for glioblastoma, characterizing seven different patient-derived cell cultures from the HGCC consortium. One-day-old zebrafish embryos were injected orthotopically with GFP-tagged glioblastoma cells, added to 96-well plates and loaded into the IncuCyte automatic imaging system, originally developed for real-time cell monitoring, with a maximum of 576 fish per instrument. Tumor development was followed every 4-6 hours up to five days post fertilization, generating up to 10 000 images per experiment. To analyze such vast image data, we developed and trained a neural network image classifier that was able to sort out valid from blurry images, identify live vs dead fish, and analyze tumor size in living animals. Tumor growth curves were established individually for every xenograft from valid images with live fish. Tumor initiation probability of different patient-derived cell lines was calculated from the number of fish in which the cell line had a positive tumor growth curve. Three out of seven cell lines had a tumor initiating capacity, showing a complete overlap with mice xenografts models using the same cell lines. U3013MG and U3054MG propagated xenograft tumors in close to 100% of the embryos, while U3180MG had a more heterogeneous tumor take. To investigate if the model can be used to distinguish patient-specific drug response, we treated xenografts with the proteasome inhibitor marizomib, currently in clinical trials for glioblastoma (NCT02903069). Indeed, zebrafish survival was extended in fish harboring the proteasome inhibitor sensitive cell line U3013MG, but not the resistant cell line U3180MG. In summary, we introduce a high-throughput *in vivo* model for drug evaluation in patient-derived glioblastoma cells, following tumors with automatic longitudinal imaging.

Discussion and future perspectives

There is an urgent need for novel treatment options for patients with high-risk neural cancers. In this thesis, I have explored different strategies to identify novel drug targets for glioblastoma and high-risk neuroblastoma. Further, I have designed follow-up studies for each target based on disease and target biology. Last, I have developed zebrafish models for high-throughput *in vivo* evaluation of novel targets. Below, I discuss the novel findings in this thesis and future implications and perspectives.

Data mining as a strategy for target identification

In paper I, we integrate large datasets from public databases to match neuroblastoma risk signatures with possible treatments and evaluate our predictions in patient-derived cell models. The new algorithm, TargetTranslator, provides a new way of linking data and find unexpected drug targets. As systematic integration of multiple data layers requires expertise in bioinformatics, these type of analyses are inaccessible for many cancer researchers. To facilitate this multistep process and to make it available for more scientists, we provide a novel tool, available on targettranslator.org, which facilitates the linking of drug targets to patient data from three cohorts of neuroblastoma and 33 different cancers from the TCGA consortium. We expect that the algorithm will be useful when analyzing other cancer types. As a relevant example, stratifying breast cancer patients only on HER2/*ERBB2* expression, TargetTranslator suggests the use of HER2 targeting compounds ($p<1.8\times10^{-6}$), which is a clinically available therapy for HER2 positive breast cancer, but also discovered unexplored links. By linking patient data to multiple databases, TargetTranslator provides a new paradigm, where targeted therapies might be suggested using computational tools.

Novel targets for neuroblastoma

In neuroblastoma, the dominating oncogene coupled to high-risk disease is N-Myc. Unfortunately, the N-Myc protein is difficult to target and as a result, many studies have been focused on targeting N-Myc indirectly, as previously discussed. Applying TargetTranslator on *MYCN*-amplified neuroblastoma

identified several novel targets. Of the targets evaluated, CNR2 and mitogen-activated protein kinase 8 (MAPK8/JNK1) were the most interesting. CNR2 is a G-coupled protein receptor and the *CNR2* gene is located on the commonly deleted part of chromosome 1p36. Activation of the target led to N-Myc protein reduction and cell death. In relapse neuroblastoma, the most recurrent alterations occur in the RAS-MAPK pathway. MAPK signaling includes three families of proteins - ERK, p38 and JNK - with specific downstream signaling events. Activation of Jun, the main transcription factor phosphorylated by JNK, has been predicted to be a part of the core regulatory circuit of neural-crest cell (NCC)-like neuroblastoma cells (Boeva et al., 2017). Both activation of CNR2 and inhibition of JNK reduced tumor growth in two xenograft models of high-risk neuroblastoma, strengthening their translational potential. A novel high-throughput zebrafish model of high-risk neuroblastoma facilitated the *in vivo* evaluation of the drug. Unfortunately, the JNK inhibitor AS601245 caused toxicity in mice, and follow up studies using other JNK inhibitors is warranted.

Patients with *ALK*-mutated neuroblastoma show a heterogeneous response to ALK inhibitors, as previously discussed. As the frequency of *ALK* mutated cases is low, the resulting signature did not correlate between our cohorts, and was not further analyzed using TargetTranslator. Interestingly, when using a gene signature approximating *ALK* mutation status in neuroblastoma (Lambertz et al., 2015), identified targets related to ALK downstream signaling (PI3K-MTOR-RPS6KB1 and RAF1), but also to AURKA and AURKB were identified. The significance of these novel links requires future studies and might help to overcome ALK inhibitor resistance in neuroblastoma.

Novel targets for glioblastoma

In paper II and III, we identified two novel drug targets - the sigma receptor and ZBTB16 – and described a two-way separation of patients into proteasome inhibitor sensitive and resistant subgroups, using functional screening in characterized patient-derived glioblastoma cell cultures. Resistant cells showed increased p53 activation, including redox regulators, and the anti-tumor effect could be inhibited by antioxidants. The vulnerability in resistant cells could be increased using drug combinations. Sensitivity could be predicted using a gene signature, which was validated in a separate cohort, strengthening our predictive pipeline. This finding has clear clinical implications as proteasome inhibitors are under evaluation in glioblastoma patients and have shown activity in some cases (Kong et al., 2018)(NCT02903069). Future studies on the clinical impact of our prediction signature of proteasome inhibitor sensitivity in patients is warranted.

Sigma receptor 1 is a chaperone mainly located in the endoplasmic reticulum (ER) in the mitochondria-associated membrane, where it regulates calcium 2+ flux to mitochondria in response to ER stress (Hayashi and Su, 2007; Mori et al., 2013). In breast cancer cells, sigma receptors bind cholesterol and modulate lipid rafts (Palmer et al., 2007). We found that glioblastoma cells are sensitive to the sigma receptor ligands. Sensitivity to sigma receptor modulation was coupled to lipid metabolism in the cells and could be synergistically potentiated by cholesterol reduction using statins, while lipid excess reduced the effect. The sigma receptor ligand rimcazole, a blood-brain penetrating compound developed for antipsychotic use, causes lipid droplet formation in the cells and induced apoptosis. This finding identified a potential treatment that was able to penetrate the blood-brain barrier and access glioblastoma cells. However, in clinical trials, rimcazole induced seizures and the development was not further pursued (CT1460/1/2). Therefore, further studies on the involvement of sigma receptors in glioblastoma should be extended to include more clinically relevant compounds. Further, the effect of lipid homeostasis on sigma receptor ligand sensitivity motivates the characterization of the glioblastoma cell metabolome as an added layer in drug sensitivity prediction.

The transcription factor ZBTB16, also named PLZF, is a novel glioblastoma candidate oncogene. We show that ZBTB16 increase proliferation and induces invasion of glioblastoma cells into surrounding tissue, a hallmark of glioblastoma. Previous genome wide association studies have identified an intronic single nucleotide polymorphism in *ZBTB16* as a risk locus for glioma development (Kinnersley et al., 2015; Melin et al., 2017). Future studies that elucidate the mechanism behind ZBTB16's regulation of glioblastoma proliferation and invasion are needed. To date, there are no available compounds for targeting ZBTB16 directly. Development of drug candidates or *in vivo* knockdown strategies, available through e.g. lipo polymeric nanoparticle infusion (Yu et al., 2017), for ZBTB16 require further investigations.

New *in vivo* models for glioblastoma treatment evaluation

In paper IV, we develop a fast xenograft model of glioblastoma in which tumor growth can be followed automatically over time. We identify three patient-derived cell cultures, two primary and one recurrent, with tumor initiating capacity in zebrafish embryos and find a varied sensitivity to proteasome inhibition, correlating with findings in paper II. In a clinical setting, tumor latency in rodent patient-derived xenografts is an obstacle for its use as patient avatars and treatment evaluation. Instead, the use of zebrafish patient-derived zebrafish xenografts could reduce the time to clinical decisions, making it a tractable option for co-clinical trials and is now under evaluation for epato-

biliar-pancreatic cancers and gastro-intestinal cancers (NCT03668418). Our study indicates that heterogeneous patient responses in glioblastoma can also be modeled in zebrafish.

In line with the 3R (replace, reduce, refine) principle of animal research, we believe that zebrafish embryo xenografts will partially replace and reduce the usage of rodent models in drug testing. This requires further characterization of the model to fully understand what phenotypes can and cannot be modeled in this system. As an example, the zebrafish has doubled its genome during evolution, and while many human genes have zebrafish equivalents, they might not function in the same way. As translational success is limited by the model systems, understanding the capacity and limitation of every model will help in making more accurate conclusions about the translational potential of a treatment.

Future perspectives

The explosion of novel techniques and multi-disciplinary collaborations has led to novel opportunities for research in tumor biology, prevention, treatment, and diagnosis. Single cell methodologies, e.g. RNAseq, have not only distinguished tumor evolution trajectories, but when linked to advanced mathematics, the rate and direction of cellular phenotype switching can be estimated (Manno et al., 2018). Applying neural network methodologies on images of hematoxylin/eosin-stained patient samples (Xu et al., 2017) and the development of high-resolution spatial transcriptomic (Vickovic et al., 2019) makes it possible for algorithms to identify the grade and histology of a tumor, which question the need for human pathologists to classify cancer in the future. Looking forward, translational research and collaborative efforts covering multiple research disciplines will be crucial for the coming research era.

Concluding remarks

Unbiased identification of novel targets in cancer require large-scale experiments and integration of data from multiple sources. In this thesis work, we have explored multiple strategies to identify novel drug targets on the gene knockdown, protein, and transcriptome level and propose several novel drug targets for high-risk neural cancers. To evaluate the novel targets, we have developed high throughput zebrafish models. Future work will involve the evaluation of CNR2 as a target in neuroblastoma, the refined use of proteasome inhibitors in glioblastoma, and will extend the characterization of ZBTB16 and sigma receptors as potential targets in glioblastoma.

Populärvetenskaplig sammanfattning

Neuroblastom och glioblastom är två dödliga former av tumörer i nervsystemet. Neuroblastom uppstår i det sympatiska nervsystemet hos små barn, vanligen i binjuren. Glioblastom uppkommer nästan alltid hos äldre personer där en mängd ackumulerade mutationer leder till att stamceller i hjärnan börjar växa ohämmat.

Man delar upp neuroblastom i riskgrupper baserat på parametrar som beskriver tumörens spridning, barnets ålder samt graden av differentiering hos tumörcellerna. Ökade mängder av proteinet N-Myc är associerad med hög risk. Hos barn med lågriskneuroblastom kan tumören försvinna av sig själv utan behandling, medan bara hälften av patienterna med högriskneuroblastom överlever 5 år efter diagnos, trots mycket intensiv behandling.

Hos glioblastompatienter är det vanligt att cancercellerna sprids till stora delar av hjärnan. Eftersom man inte kan komma åt alla cancerceller med kirurgi eller intensiv strålning, då detta skulle skada för stor del av den normala hjärnvävnaden, blir glioblastom svårbehandlat. Cellgiftsbehandling förlänger vanligtvis livet med någon månad och med bästa möjliga behandlingen, som omfattar både kirurgi, strålning och cellgifter, är överlevnaden endast 15 månader hos snittpersonen. Det finns ett stort behov av nya och effektiva behandlingar mot neuroblastom och glioblastom. I den här avhandlingen har vi identifierat nya möjliga behandlingar mot både neuroblastom och glioblastom.

I **delarbete I** har vi utvecklat en ny algoritm, TargetTranslator, som kan förutsäga vilka läkemedel som har potential att vara aktiva mot neuroblastom. Metoden analyserar först vilka gener som är påslagna (uttryckta) i cancercellerna hos högriskpatienter. Därefter söker algoritmen i databaser som beskriver hur läkemedel ändrar geners uttryck. På så vis kan vi förutsäga vilka läkemedel som borde ändra genuttrycket i tumörcellerna på ett positivt sätt. Vi testade tolv av de läkemedel som algoritmen föreslog på celler som är framodlade från patienter med högriskneuroblastom. Resultaten visade att de förutsagda läkemedlen minskade mängden N-Myc-protein i cellerna och ökade celldöden. Efter behandling med två av läkemedlen minskades tumörstorleken både i en zebrafiskmodell och i en musmodell med neuroblastom. Ett av läkemedlen inaktiverar proteinet MAPK8 och det andra aktiverar proteinet CNR2. Vår slutsats är att dessa två läkemedel är möjliga nya behandlingsalternativ mot neuroblastom.

I **delarbete II** beskrivs ett annat sätt att hitta nya behandlingar mot glioblastom. Här undersöktes effekten av mer än 1200 läkemedel på celler

tagna från 100 patientbiopsier. Vi fann att patientcellerna var olika känsligheten mot en grupp av läkemedel som hämmar proteasomer. Proteasomer är strukturer i celler som bryter ner proteiner. Det finns redan pågående kliniska utredningar om effekten av dessa läkemedel i patienter med glioblastom, där vissa patienter svarar bättre på behandlingen. Om man inte kan särskilja patienter med den känsliga varianten av glioblastom kan det innebära att effekten av läkemedlet missas i den kliniska studien. Vi fann att känsliga celler inte kunde hantera höga halter av reaktiva syreföreningar och att detta var kopplat till funktionaliteten hos tumörsuppressorn p53, en gen som vanligen skyddar mot cancer. I ett separat försök med celler från ett annat forskningsinstitut kunde vi förutsäga känsligheten för proteasomhämmarna baserat på tumörens genprofil. I screenen fann vi även andra kopplingar mellan läkemedelskänslighet och molekylära signaturer och kunde validera en koppling mellan känslighet för sigmareceptorligander och cellens fettmetabolism.

I **delarbete III** testade vi ifall glioblastomcellerna var beroende av olika gener för att överleva genom att slå ut genen med siRNA, en metod som minskar uttrycket av en gen. Vi identifierade transkriptionsfaktorn ZBTB16 som en onkogen (gen som kan bli cancerfrämjande) i glioblastom. ZBTB16 påskyndar celldelningen och ökar glioblastomcellernas invasion i hjärnan på zebrafiskyngel. Minskandet av ZBTB16 med siRNA ledde till ökad celldöd. Sammanfattningsvis identifierade vi i delarbete II och III ett antal nya möjliga målproteiner som kan lägga grunden för nya behandlingar av glioblastom.

För att utveckla nya läkemedel som ska användas i människor krävs idag omfattande försök på djur, vanligtvis möss. Dessa försök är resursintensiva och ger begränsad information om hur läkemedel påverkar cancercellerna över tid. I **delarbete IV** har vi utvecklat en ny modell för läkemedelsutvärdering i transparenta zebrafiskembryon. Grönfluorescerande glioblastomceller från 7 olika patienter injiceras i hjärnan hos zebrafiskembryon. Utvecklingen av tumörerna kan sedan observeras med hjälp av automatisk bildbehandlingsteknik. Bilderna analyseras med artificiell intelligens för att förstå hur läkemedel påverkar tillväxten av tumörerna, specifikt för varje patients celler. Cellerna injicerades även i embryon med rött kärlsystem för att kunna följa hur tumörcellerna interagerar med blodkärlen, då glioblastom karakteriseras av onormala kärl. Denna studie kartlägger hur glioblastomceller från olika patienter växer i hjärnan på zebrafiskembryon. De nya modellerna kan användas för utvärdering av nya läkemedelskandidater.

Sammanfattningsvis ger denna avhandling en ökad repertoar av målmolekyler som kan användas för behandling av de dödliga cancerformerna neuroblastom och glioblastom. De nya målmolekylerna behöver fortsatt utvärderas för att klargöra funktionen i cancercellerna och avgöra potentialen av dem som behandlingsmål. Som en del i detta har vi utvecklat en ny glioblastommodell där tumörcellerna kan följas i realtid i hjärnan på fiskembryon.

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