General Introduction

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Tumors that originate in the human brain are called primary brain tumors. Distinct subtypes are recognized by the World Health Organization (WHO), as distinct types of brain tissue or anatomic location can give rise to specific tumors. One of them is called glioma, named so as it is hypothesized that this type of tumor arises from glial cells (supporting tissue of the brain). Although it is the most common type of primary malignant brain tumors in human, it is a rare disease with an incidence rate of approximately 6 per 100.000 persons annually in Europe and the United States.[1, 2] Extrapolated to the Dutch situation, this means approximately 1000 persons per year in the Netherlands are newly diagnosed with a glioma.

CLASSIFICATION AND PROGNOSIS OF DIFFUSE GLIOMAS

Diffuse gliomas have a variable prognosis with overall survival rates ranging from only several months to more than 20 years, depending on the subtype.[3, 4] It is clear that very aggressive tumors with an overall survival of only a few months need a different treatment strategy than more indolent tumors with an overall survival of multiple years. Therefore, classifying gliomas into different subtypes that reflect their clinical behavior, prognosis and/or response to treatment is essential.

Gliomas are classified according to the WHO classification of tumors of the central nervous system and traditionally this was based on histological features.[5] However, differences between histological subtypes on microscopic level can be very subtle, and therefore this classification was subject to substantial interobserver variability.[6-8] This potentially results in suboptimal treatment of some patients which is undesirable. The WHO classification scheme was updated in 2016 following many observations that showed better discrimination of clinically relevant subclasses of glioma by classifying on the molecular background of brain tumors.[5] The updated WHO classification now consists of both histologic and molecular features and this has led to marked improvement of objectivity and prognostic significance. Cornerstone of the WHO 2016 classification is testing for presence of mutations in isocitrate dehydrogenase gene 1 or 2 (*IDH1/2*) and presence of a combined deletion (co-deletion) of chromosomal arms 1p and 19q. Based on just these two markers, three subtypes of diffuse lower grade glioma can be recognized; 1) Oligodendroglioma, *IDH1/2* mutant and 1p/19q co-deleted (*IDH1/2* mutation in combination with presence of a co-deletion of the entire 1p and 19q chromosomal arms); 2) Astrocytoma, *IDH1/2* mutated (*IDH1/2* mutation without 1p19q co-deletion); and 3) Astrocytoma, *IDH1/2* wildtype. The highest grade of glioma, glioblastoma, is separated in *IDH1/2* mutated and *IDH1/2*

wildtype (most common form).[5, 9] Molecular aberrations described in *IDH* wildtype glioblastoma are generally equal to the aberrations described in *IDH* wildtype astrocytomas and the outcome is similarly poor (median survival approximately 15 months). Hence, low-grade and anaplastic *IDH* wildtype astrocytomas are often considered as misdiagnosed glioblastoma. Oligodendrogliomas and *IDH* mutated astrocytomas have a much better prognosis with a median overall survival of 12-14 years and 3-8 years respectively. Next to *IDH* gene mutations and 1p19q co-deletion, there are many other frequently reported genetic changes in glioma that are not used for classification criteria, but which can support the diagnosis. For example, *TP53* and *ATRX* mutations are frequently reported in *IDH* mutated astrocytoma. These two mutations are mutually exclusive with 1p/19q co-deletions in glioma. *CIC* and *FUBP1* mutations are frequently reported in *IDH* mutant 1p19q co-deleted oligodendroglioma, but almost never in *IDH* mutated or wildtype astrocytoma. *TERT* promotor mutations are present in almost all *IDH* mutant 1p19q co-deleted oligodendrogliomas and are frequently reported in *IDH* wildtype astrocytoma and glioblastoma, but in principle not in *IDH* mutated astrocytoma.[9-12] Also, mutations or amplifications of the *EGFR* gene are frequently reported, mostly in *IDH* wildtype glioblastoma. Observation of this aberration can support diagnosis, but is not related to prognosis. For a detailed description of the WHO 2016 classification scheme, see Figure 1.

Apart from classification of diffuse gliomas into histomolecular subgroups, diffuse gliomas are also graded (grade II, III, or IV) to further stratify the aggressiveness of the tumors. This is currently still based on the presence of the following histopathological features: nuclear atypia, mitotic activity, microvascular proliferation, and necrosis.[13] Unfortunately, grading of glioma is subject to interobserver variability as scoring of these histological criteria may be difficult due to tumor heterogeneity, small sample volumes, and different interobserver judgement. Therefore, although the updated classification outflanks the previous version for prognosis estimation, there is still variation in prognosis of patients within the major glioma groups. Further improvement and refinement of the classification would be very welcome, especially with markers that reflect aggressiveness/grade within the current WHO subgroups, but so far no molecular markers have been identified that aid in objective grading. **Chapter 2, 3, and 4** of this thesis focus on the efforts to further refine the WHO classification and are described briefly in the last paragraph of this chapter.

GLIOMA TREATMENT

Diffuse gliomas have an infiltrative growth pattern and are often located in or near eloquent areas of the brain (i.e. the sensory cortex, motor cortex, basal ganglia, and

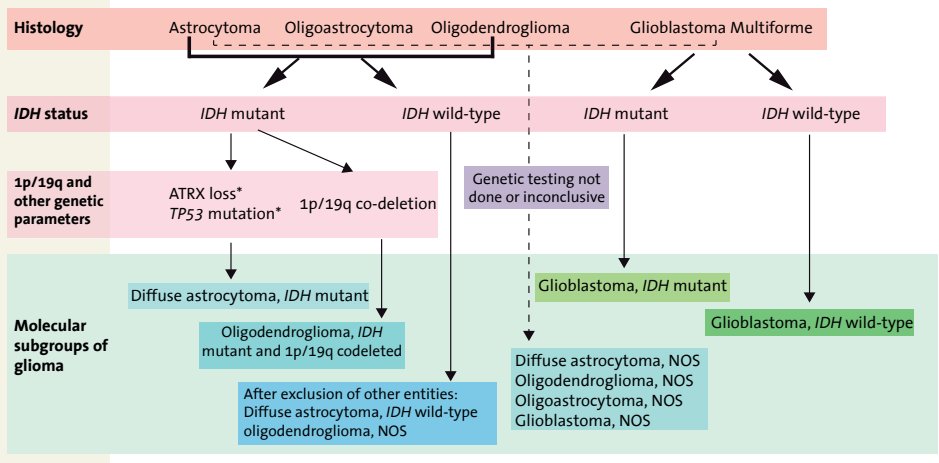

Figure 1. 2016 WHO classification scheme of diffuse glioma. Figure adapted (with permission) from Louis et al.[5]

language/speech area).[4] Therefore it is impossible to fully resect a glioma. As our knowledge on the molecular background of glioma improves, much research nowadays focusses on targeting glioma specific mutations and developing glioma specific immunotherapies. So far this has not led to new standard therapies in daily clinical setting. Therefore, the common available modalities for glioma treatment still are (a combination of) surgical resection, chemotherapy, and radiotherapy.[3, 4, 14-18] How to best employ these different treatment modalities remains a matter of controversy. In individual patients the combination, timing, and sequence is often decided based upon the perception of prognostic factors within a specific patient, such as the clinical condition, location and size of the tumor, and the integrated WHO 2016 diagnosis which is assessed following surgery. The intent of surgery is threefold; to provide tissue for diagnostic purposes (histology and molecular testing), to remove as much tumor as possible to relieve symptoms and to improve survival. Whether that latter objective is actually realistic in low grade glioma has been a topic of debate for years. In the past a so called wait-and-scan approach was the common strategy to treat a lesion suspected for low-grade glioma.[19, 20] This strategy consists of monitoring tumor behavior over time with regular interval MRI scans, with the intention to start active treatment once significant growth of the lesion, clinical deterioration or malignant transformation (signs of contrast enhancement on brain imaging) has occurred. The rationale behind this was the incurable nature of these tumors, the low growth rates and the fact that patients usually present with minor symptoms, such as controllable seizures. Furthermore, the fear for inducing neurological deficits by a neurosurgical procedure withheld many neurosurgeons from aggressive surgical treatment. Performing early surgery on these lesions was therefore generally seen as inappropriate,

as surgery comes with these risks and is not curative. This consensus on treatment of low-grade glioma patients gradually changed in the past decade towards a standard of care where clinicians aim for aggressive resections as early as possible when this is safely possible. This was due to the growing evidence that early and extensive resections are associated with a better clinical outcome (longer overall survival) and the improvement of surgical techniques that allow more safe and extensive resections.[21-26] However, all studies investigating the role of surgery for low grade glioma are retrospective, and are therefore exposed to certain indication and selection bias. Nonetheless, as a prospective study to answer this question is generally considered not feasible for various reasons, retrospective evidence for early and extensive resections is the best option and over time early resection has become part of the international guidelines on glioma treatment. Nevertheless, the timing and extent of resection remain topics of debate in the field. **In chapter 5 and 6** we focus on this still timely topic.

SCOPE OF THIS THESIS

This thesis mainly focusses on lower grade diffuse gliomas (grade II and III). Although the objectivity and prognostic value of glioma classification have improved with the updated WHO classification, further refinement in order to achieve more efficient treatment strategies is mandatory. In **chapter 2** we analyze the publically available whole exome sequencing data of *The Cancer Genome Atlas* (TCGA) of both low and high grade glioma, to find additional prognostic markers within WHO recognized glioma subgroups. In **chapter 3** we report the prognostic relevance of additional mutations and copy number alterations in *IDH* mutated grade II glioma, using a targeted next generation sequencing panel that is also used in routine diagnostic setting. In **chapter 4** we report on a relatively large group of *IDH*-wildtype gliomas, and show this is in fact a molecular and clinical heterogeneous group of tumors. As mentioned above, the role of surgery for lower grade gliomas has been controversial in the past. Consensus in the field shifted from a wait-and-scan approach to early and aggressive resection during the last decade. As the WHO classification of gliomas has been completely revised and is now predominantly based on molecular criteria, the impact of extent of resection needed to be re-evaluated in molecularly defined low grade glioma which we describe in **chapter 5.** In **chapter 6**, we focus on the timing of surgery and the impact on outcome in presumed low-grade glioma, but with a set-up wherein we tried to minimize the above mentioned indication and selection bias as much as possible. In **chapter 7**, we provide insight in the location distribution of specific WHO molecular subgroups of glioma in the human brain. Finally, **chapter 8** discusses the main findings of chapters 2 to 7 and puts this in perspective with recent literature and opinions in the field.

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