In this thesis ‘molecular characterization of low-grade glial neoplasms’ we have sought for markers that are informative for the disease course of patients with diffuse low-grade glioma (LGG) and improve the classification of low-grade glioneuronal tumors (GNT).

Diffuse glioma are brain tumors originating from the supporting tissue of the central nervous system. LGG are classified as WHO grade II diffuse glioma and are most commonly diagnosed in adults during their 3rd or 4th decade in life (155). There is no cure for these patients and during the disease course the recurrent tumors often evolve to a higher malignancy grade. The median survival of patients with an LGG is six years. Still, there is a wide variation in survival (less than two to more than twenty years). The favorable impact of surgery on survival is established, whereas the role of irradiation and the role of chemotherapy is not yet clear. Side effects such as cognitive deficits may develop in the long-term, and require accurate timing of these postoperative treatments (148). The use of parameters with prognostic value can prevent or delay the occurrence of such side effects; patients with an aggressive tumor and an expected short survival should receive adjuvant treatments in an early stage to stall tumor growth, while in patients with indolent LGG watchful waiting (i.e. frequent control by MRI scans) seems a better strategy. Despite the identification of various clinically prognostic factors, such as age at time of diagnosis, histological subtype and extent of resection, there is still a strong need for better differentiation in expected survival. Molecular markers with prognostic value could be very helpful to achieve this. Predictive markers, on the other hand, are of use in decision making with regard to the type of treatment. The term ‘predictive’ relates to increased or reduced efficacy of treatment based on the presence or absence of a molecular marker.

The first chapter of this thesis addresses pharmacogenomics and –genetics in glial oncology (304). The chapter begins with a historical overview of predictive markers in several types of tumors. The chapter continues with an explanation of the diagnostic and therapeutic challenges for patients with diffuse glioma (WHO grade II-IV) and provides an overview of the state of the art of molecular markers with prognostic and predictive significance in diffuse glioma in 2013.

The second and third chapter contain studies focused on the evolution of LGG; molecular markers with prognostic value and spatial and temporal heterogeneity (175;184). A well-known and diagnostically implemented molecular marker for diffuse glioma is a combination of the loss of the short arm of chromosome 1 (1p) and of the long arm of chromosome 19 (19q); the 1p/19q co-deletion. Chromosomal aberrations are parts of the genome present in surplus (gain) or at a lower frequency (deletion/loss) compared to the normal diploid state of DNA. LGG with 1p/19q co-deletion have a less aggressive behavior than those with similar histology but lacking the co-deletion. Mutation of IDH is also associated to a favorable prognosis. These two markers, however, are insufficient to optimally stratify all patients with LGG for different therapeutic strategies. In chapters 2 and 3, two independent, retrospectively collected cohorts of 126 and 98 patients with LGG served in alternating fashion as study and validation cohort, supplemented by a confirmation cohort of 184 patients in chapter 3. Detailed clinical data, including survival time were noted and DNA was isolated from the surgical specimens. Genome-wide analysis for chromosomal aberrations was applied using two techniques, array comparative genomic
hybridization on fresh frozen material in chapter 2 and shallow genome wide sequencing on FFPE material in chapter 3. Chromosomal aberrations detected in the respective cohorts were analyzed for their association to survival. In addition to the previously established favorable 1p/19q co-deletion, three chromosomal aberrations associated with an unfavorable prognosis; losses of 11p and 19q (independent of 1p loss) in chapter 2 and distal loss of 10q in chapter 3.

Visual evaluation of the chromosomal copy number profiles generated in chapter 3 resulted in the hypothesis that chromosomal aberrations might be present in only a part of the tumor. This phenomenon is referred to as ‘spatial heterogeneity’. Sixty-five spatially distinct surgical samples of 17 LGG were analyzed for variability in chromosomal aberrations across each tumor. Spatial heterogeneity was observed in the tumors of fifteen of these 17 patients with LGG, and 68% of the total number of chromosomal aberrations was not homogeneously detected. This variability in molecular features could not be associated to histological features. Spatial heterogeneity implies that the tumor is evolving in time. In order to study temporal evolution we therefore collected 24 recurrent tumors of 20 patients to analyze chromosomal aberrations in these samples. Almost 40% of the chromosomal aberrations of this subgroup of paired initial and recurrent tumors were unique to the recurrent tumor. Distal 10q loss was heterogeneously detected in spatially distinct samples of the initial tumors and was a frequently occurring new chromosomal aberration in recurrences, commensurate with it unfavorable prognostic value. This combination of results suggests that distal 10q loss is a late-onset molecular event in subclones of LGG which are involved in tumor progression.

There is no standardized postoperative regimen for patients with LGG with, or at risk for, tumor progression. Irradiation prolongs overall survival, while two regimens of chemotherapy TMZ and PCV (procarbazine, CCNU and vincristine) are, so to speak, competing to set foot in treatment protocols. Both are beneficial for patients with high-grade glioma, and their effect in LGG is currently under investigation in clinical trials (206;322;332). Preliminary reports at conferences suggest that PCV + irradiation prolongs overall survival, compared to irradiation only (305). TMZ, on the other hand, is not superior to irradiation, but has not been tested in combination with irradiation (332). TMZ employs futile cycling of DNA repair mechanisms in order to ultimately induce apoptosis of tumor cells. Chapter 4 focuses on a potential mechanism of therapeutic resistance of LGG to TMZ (324). Molecular alterations of a unique cohort of 34 patients with initial LGG and paired recurrent tumors were studied. Ten patients of this cohort were treated with temozolomide after surgery of the initial tumor. In the recurrent tumors of six of these ten patients a very high number of molecular alterations was detected, referred to as temozolomide-associated hypermutation, because the type of alterations can be assigned to the specific damaging effect of this agent. All recurrent tumors of this subgroup were classified as secondary glioblastoma (WHO grade IV), compared to variable histological entities in recurrent tumors of patients treated with TMZ without hypermutation and patients not treated with TMZ. The samples were analyzed for exome-wide mutations, chromosomal aberrations, and MGMT methylation levels. The activity of the DNA repair gene MGMT is negatively influenced by increased promoter-methylation levels. In the recurrent tumors with the temozolomide-associated hypermutation profile, DNA repair was consistently compromised by (a combination of) mutations in DNA repair genes, chromosomal loss of genomic regions covering DNA repair genes and significantly elevated methylation levels of MGMT.
No consistent characteristic of the matched initial tumors could explain why hypermutation occurs in only a subset of the temozolomide treated patients. Chromosomal losses covering DNA repair genes were already detected in two initial tumors, hence we can not exclude that these tumors were susceptible to hypermutation prior to temozolomide administration. The clinical relevance of temozolomide-associated hypermutation remains unclear and requires evaluation in larger cohorts. Of interest is a potential benefit for a hypermutated tumor for increased efficacy of immunotherapy (337). Altogether, the mutagenic effect of temozolomide on DNA repair genes seems to be involved in the potential resistance mechanism to this agent in a subset of patients.

The advent of shallow whole genome sequencing enabled a new approach to the analysis of chromosomal aberrations in archival material, and has proven instrumental for the discovery of the unfavorable distal 10q loss in LGG (154). After optimizing the technique with these LGG samples it has been applied in chapter 5 in GNT (279). GNT are rare, mainly benign tumors that often cause epilepsy in children and young adults. Surgery is performed if the epilepsy is insufficiently treated with medication and the epileptic focus needs to be removed. GNT encompass various histological subgroups (155;156). Inter-observer variability among pathologists can be explained by the variable histology in these tumors and the lack of unequivocal criteria for diagnosing neoplasms that do not show the prototype histology. Also, some GNT are difficult to distinguish from LGG because they show a similar diffuse growth pattern. In chapter 5 chromosomal aberrations in a large cohort (n=114) of GNT are studied to gain insight into their molecular variability and contribute to the development of more objective classification including molecular features. Despite selection of only the two most frequent histological subgroups of GNT for this study, low-grade ganglioglioma and dysembryoplastic neuroepithelial tumor, there was indeed substantial variability in histological features. Unfortunately, chromosomal aberrations were also highly variable in this cohort and the study did not result in a classification superior to histopathological analysis since none of the chromosomal aberrations associated with histological features or clinically relevant parameters. Studies focusing on a new classification of GNT may require investigation at different molecular levels such as epigenetic dysregulation (328). What we did learn is that gain of chromosomes 5 and/or 7 are the most frequently present chromosomal aberrations irrespective of histological subgroup, suggesting a shared origin for these tumors. Comparison of a subgroup of LGG to dysembryoplastic neuroepithelial tumors that are histologically difficult to distinguish identified a possible discriminative value for gain of whole chromosome 5 which was only present in the latter group. The spatial heterogeneity for chromosomal aberrations as observed in the LGG cohort of chapter 3 may also be present in the GNT as similar patterns were observed. Heterogeneity can be associated to tumor evolution and is of specific interest to study the molecular biology of low-grade GNT which are considered to be indolent tumors, and only rarely progress.

This thesis was composed in a time of important genomic discoveries for oncology in general. The progress made can be attributed to technical innovations that enabled comprehensive, affordable studies of molecular alterations in large cohorts of samples. Furthermore, the opportunity was exploited to apply such techniques to a collection of well-annotated archival samples with detailed clinical follow up data of the patients. The studies presented in this thesis contribute to unravel molecular features of
low-grade glial neoplasms and at the same time raise new questions that form the basis for ongoing and future research.