Glioma Through the Looking GLASS: the Glioma Longitudinal Analysis consortium, molecular evolution of diffuse gliomas

The GLASS consortium*

*A list of participants and affiliations appears at the end of the paper.

Corresponding authors:

Roel Verhaak
roel.verhaak@jax.org

Mustafa Khasraw
mustafa.khasraw@ctc.usyd.edu.au

20 **AUTHORLIST**
21 1. Kenneth Aldape, MD, Department of Laboratory Medicine and Patholobiology
22 University of Toronto, 101 College St, Toronto, ON, Canada M5G 1L7
23 2. Samirkumar B Amin, MBBS, Ph.D., The Jackson Laboratory for Genomic
24 Medicine, Ten Discovery Drive, Farmington, CT, 06032 USA.
25 3. David M Ashley, MBBS(Hon), FRACP, PhD, Preston Robert Tisch Brain Tumor
26 Center at Duke, Duke University Medical Center, Durham, North Carolina 27710
27 USA.
28 4. Jill S Barnholtz-Sloan, PhD, Case Comprehensive Cancer Center, Case Western
29 Reserve University School of Medicine, 2103 Cornell Rd, WRB 2-526, Cleveland,
30 Ohio 44106 USA.
31 5. Amanda J Bates, National Brain Tumor Society, 55 Chapel Street, Suite 200,
32 Newton, MA 02458 USA.
33 6. Rameen Beroukhim, MD PhD, Departments of Medical Oncology and Cancer
34 Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA
35 7. Christoph Bock, PhD, CeMM Research Center for Molecular Medicine of the
36 Austrian Academy of Sciences, Lazarettgasse 14, 1090 Vienna, Austria
37 8. Daniel J Brat, MD PhD, Department of Pathology, Feinberg School of Medicine,
38 Northwestern University, 300 East Superior Street, Chicago, IL 60611 USA
39 9. Elizabeth B Claus, MD PhD, Yale School of Public Health, New Haven CT 06511
40 and Department of Neurosurgery, Brigham and Women's Hospital, Boston, MA
41 02115 USA.
42 10. Joseph F Costello, PhD, The University of California San Francisco, San
43 Francisco, CA 94158 USA.
44 11. John F de Groot, MD, The University of Texas - MD Anderson Cancer Center;
45 1515 Holcombe Blvd, Houston, TX 77030 USA
46 12. Gaetano Finocchiaro, MD, Fondazione IRCCS Istituto Neurologico Besta, via
47 Celoria 11, 20133 Milano, Italy
48 13. Pim J French, PhD, Department of Neurology, Erasmus MC, 's-Gravendijkwal
49 230, 3015 CE Rotterdam, The Netherlands
50 14. Hui K Gan, MBBS, PhD, Olivia Newton-John Cancer Research Institute, Austin
51 Health, Heidelberg 3084, Melbourne, Victoria, Australia
52 15. Brent Griffith, MD, Department of Radiology, Henry Ford Health System, Detroit,
53 MI 48202 USA.
54 16. Christel C Herold-Mende, PhD, Div. Experimental Neurosurgery, Dpt
55 Neurosurgery, University of Heidelberg, INF 400, 69120 Heidelberg, Germany
56 17. Craig Horbinski, MD PhD, Department of Pathology, Feinberg School of
57 Medicine, Northwestern University, 300 East Superior Street, Chicago, IL 60611
58 USA
59 18. Antonio Iavarone, MD, Department of Neurology, Department of Pathology,
60 Institute for Cancer Genetics, Columbia University Medical Center, 10032 New York
61 USA.
62 19. Steven N Kalkanis, MD, Department of Neurosurgery, Henry Ford Health
63 System, Detroit, MI 48202 USA.
64 20. Konstantina Karabatsou, MD, Salford Royal Hospital, Stott Lane, Greater
65 Manchester, M6 8HD, U.K.
66 21. Hoon Kim, PhD, The Jackson Laboratory for Genomic Medicine, Ten Discovery
67 Drive, Farmington, CT, 06032 USA.

68 22. Mathilde CM Kouwenhoven, MD PhD, Department of Neurology, VU University
69 Medical Center/Brain Tumor Center Amsterdam, De Boelelaan 1117, 1081 HV
70 Amsterdam, The Netherlands
71 23. Kerrie L McDonald, PhD, Cure Brain Cancer Biomarkers and Trasnlational
72 Research Group, Prince of Wales Clinical School, University of NSW
73 24. Hrvoje Miletic, MD PhD, Department of Pathology, Haukeland University
74 Hospital, Bergen, Norway
75 25. Do-Hyun Nam, MD PhD, Department of Neurosurgery, Samsung Medical
76 Center, Sungkyunkwan University School of Medicine, Seoul, Korea
77 26. Ho Keung Ng, MD, Department of Pathology, Chinese University of Hong Kong,
78 Prince of Wales Hospital, Shatin, Hong Kong
79 27. Simone P Niclou, PhD, Luxembourg Institute of Health, NorLux Neuro-Oncology
80 Laboratory; 84, Val Fleuri, 1526 Luxembourg, Luxembourg
81 28. Houtan Noushmehr, PhD, Department of Neurosurgery, Henry Ford Health
82 System, Detroit, MI 48202 USA.
83 29. D Ryan Ormond, MD, Department of Neurosurgery, University of Colorado
84 School of Medicine, 12631 E. 17th Avenue, Mail Stop C307, Aurora, CO 80203 USA
85 30. Laila M Poisson, PhD, Department of Neurosurgery, Henry Ford Health System,
86 Detroit, MI 48202 USA
87 31. Guido Reifenberger, MD, PhD, Department of Neuropathology, Heinrich Heine
88 University Duesseldorf, Medical Faculty, Moorenstr 5, D-40225 Duesseldorf,
89 Germany
90 32. Federico Roncaroli, MD, Division of Neuroscience & Experimental Psychology,
91 Faculty of Biology, Medicine & Health, University of Manchester, UK Oxford Road
92 M23 9PT
93 33. Jason K Sa, PhD, Research Instittute for Future Medicine, Samsung Medical
94 Center, Seoul, Korea
95 34. Peter AE Sillevis Smitt, MD PhD, Department of Neurology, Erasmus MC, 's-
96 Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands
97 35. Marion Smits, MD PhD, Department of Radiology and Nuclear Medicine,
98 Erasmus MC, 's-Gravendijkwal 230, 301 CE Rotterdam, the Netherlands
99 36. Camila F Souza, PhD, Department of Neurosurgery, Henry Ford Health System,
100 Detroit, MI 48202 USA.
101 37. Ghazaleh Tabatabai, MD PhD, Interdisciplinary Division of Neuro-Oncology,
102 Departments of Neurology & Neurosurgery, Unviersity Hospital Tübingen
103 38. Erwin G Van Meir, PhD, Departments of Neurosurgery & Hematology and
104 Medical Oncology, School of Medicine and Winship Cancer Insitute, Emory
105 University, 1365C Clifton Rd. NE., Atlanta, GA30322, USA
106 39. Roel GW Verhaak, PhD, The Jackson Laboratory for Genomic Medicine, Ten
107 Discovery Drive, Farmington, CT, 06032 USA.
108 40. Colin Watts, MD PhD, Department of Clinical Neurosciences, Division of
109 Neurosurgery, University of Cambridge, UK.
110 41. Pieter Wesseling, MD PhD, Department of Pathology, VU University Medical
111 Center/Brain Tumor Center Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam,
112 The Netherlands, and Princess Máxima Center for Pediatric Oncology + University
113 Medical Center Utrecht, The Netherlands
114 42. Adelheid Woehrer, MD, PhD, Institute of Neurology, Medical University of
115 Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria
116 43. WK Alfred Yung, MD, UT MD Anderson Cancer Center, Houston, TX 77030
117 USA.

3

118   44. Christine Jungk, MD, Dpt Neurosurgery, University of Heidelberg, INF 400,
119   69120 Heidelberg, Germany
120   45. Ann-Christin Hau, PhD, Luxembourg Institute of Health, NorLux Neuro-Oncology
121   Laboratory; 84, Val Fleuri, 1526 Luxembourg, Luxembourg
122   46. Eric van Dyck, PhD, Luxembourg Institute of Health, NorLux Neuro-Oncology
123   Laboratory; 84, Val Fleuri, 1526 Luxembourg, Luxembourg
124   47. Bart A Westerman, PhD, Brain Tumor Center Amsterdam, Cancer Center
125   Amsterdam, VU Medical Center, Amsterdam, the Netherlands
126   48. Julia Yin, BSc, Cure Brain Cancer Biomarkers and Trasnlational Research
127   Group, Prince of Wales Clinical School, University of NSW, Australie
128   49. Olajide Abiola, , The Jackson Laboratory for Genomic Medicine, Ten Discovery
129   Drive, Farmington, CT, 06032, USA.
130   50. Mustafa Khasraw, MBChB, MRCP, FRACP, MD, The University of Sydney,
131   NSW 2006 Australia
132   51. Erik P Sulman, MD, PhD, UT MD Anderson Cancer Center, Houston, TX 77030
133   52. Andrea M Muscat, BSc(Hons), Deakin University, Geelong, VIC. 3220 Australia

4

134 **Abstract**

135 Adult diffuse glioma are a diverse group of intracranial neoplasms

136 associated with a disproportional large number of productive life years

137 lost, thus imposing a highly emotional and significant financial burden on

138 society. Patient death is the result of an aggressive course of disease

139 following diagnosis. The Cancer Genome Atlas and similar projects have

140 provided a comprehensive understanding of the somatic alterations and

141 molecular subtypes of glioma at diagnosis. However, gliomas undergo

142 significant molecular evolution during the malignant transformation. We

143 review current knowledge on genomic, epigenomic and transcriptomic

144 abnormalities before and after disease recurrence. We outline an effort

145 to systemically catalogue the longitudinal changes in gliomas, the

146 Glioma Longitudinal Analysis Consortium. The GLASS initiative will

147 provide essential insights into the evolution of glioma towards a lethal

148 phenotype with the potential to reveal targetable vulnerabilities, and

149 ultimately, improved outcomes for a patient population in need.

150

1. Introduction

151

152 Diffuse gliomas are the most frequent malignant primary brain tumors in adults.[1]

153 Almost all relapse despite intense treatment with surgery, radiation, and

154 chemotherapy. The most common and most aggressive gliomas, glioblastoma

155 (GBM), are IDH-wildtype and classified as World Health Organization (WHO) grade

156 IV. They are characterized by a median overall survival that has remained static at

157 around 15 months for decades even in selected clinical trial populations.[2-5] Patients

158 with lower-grade (WHO grades II and III) IDH mutated gliomas have a more

159 favorable prognosis but their tumors are also lethal, as they too recur and become

160 resistant to therapy.[1] The standard of care for infiltrative/ diffuse gliomas is maximal

161 safe resection, followed by chemoradiation.[6] Patients are then monitored for disease

162 progression by imaging at regular intervals following surgery. Evaluation of disease

163 progression is commonly guided by specific criteria (e.g. Response Assessment in

164 Neuro-Oncology (RANO)),[7] which rely on visual evaluation of contrast enhancement

165 and the non-enhancing hyperintense area on T2-weighted imaging. Radiologic

166 features sometimes do not distinguish between true tumor progression and its

167 imaging mimicker, pseudo-progression, and disease progression cannot be reliably

168 established based on imaging alone. Inaccurate assessment can result in premature

169 withdrawal from a specific treatment or to continue an ineffective therapy. A further

170 challenge in particular in the monitoring of lower-grade gliomas is the prediction of

171 malignant transformation without another surgical intervention leading to a new

172 histological analysis. At present, this is based on clinical progression and rather

173 imprecise imaging signs such as contrast enhancement in a previously non-

174 enhancing lesion or increase in size of measurable abnormalities, that are better

175 indicators of existing imaging features rather than impending malignant

176 transformation.

177 Molecular characterization of gliomas in recent years has advanced our

178 understanding of their genesis and has identified abnormalities that allow a better

179 classification and may be therapeutically targetable[8]. Through efforts by the Cancer

180 Genome Atlas (TCGA),[9-14] the genomes of over 1,100 grades II–IV gliomas have

181 been characterized in detail, with other groups contributing further important results

182 and datasets.[15-20] This wealth of information has provided a detailed molecular

183 portrait of primary glioma. The atlas, by design, focused on untreated tumors. The

6

184   next frontier in glioma genomics is to understand recurrent disease. This is

185   important, as patients generally die from tumor regrowth after therapy that becomes

186   increasingly more resistant. Recent reports on pilot sets of paired tumors obtained

187   before and after therapy show that there are many differences between the primary

188   neoplasm at diagnosis and the recurrent tumor.[21] Malignant progression of gliomas,

189   similar to other cancers, is the result of an evolutionary process that involves

190   reiterative cycles of clonal expansion, genetic diversification, and clonal selection

191   under microenvironmental pressures, including overcoming antitumor immune

192   responses.[22] The presence of multiple cell populations with an array of different

193   mutations is at least partly responsible for rapid induction of intrinsic resistance to

194   therapy in gliomas.[23] Adaptive epigenetic and phenotypic responses are equally

195   important. The emerging understanding of this dynamic evolution of the glioma

196   genome has major implications for cancer biology research and potential

197   development of effective therapy. However, like the molecular landscape of primary

198   tumors, a full understanding of the dynamic evolution of gliomas there can only be

199   achieved through (a) profiling of sufficiently large patient cohorts to achieve statistical

200   significance and (b) standardization across biospecimen processing and data

201   platforms. The Glioma Longitudinal AnalySiS (GLASS) consortium has been initiated

202   to generate a comprehensive molecular and radiological portrait from paired primary

203   and recurrent adult gliomas, including clinical patient data, to enable discovery of

204   vulnerabilities that render the tumor sensitive to therapeutic intervention (Figure 1).
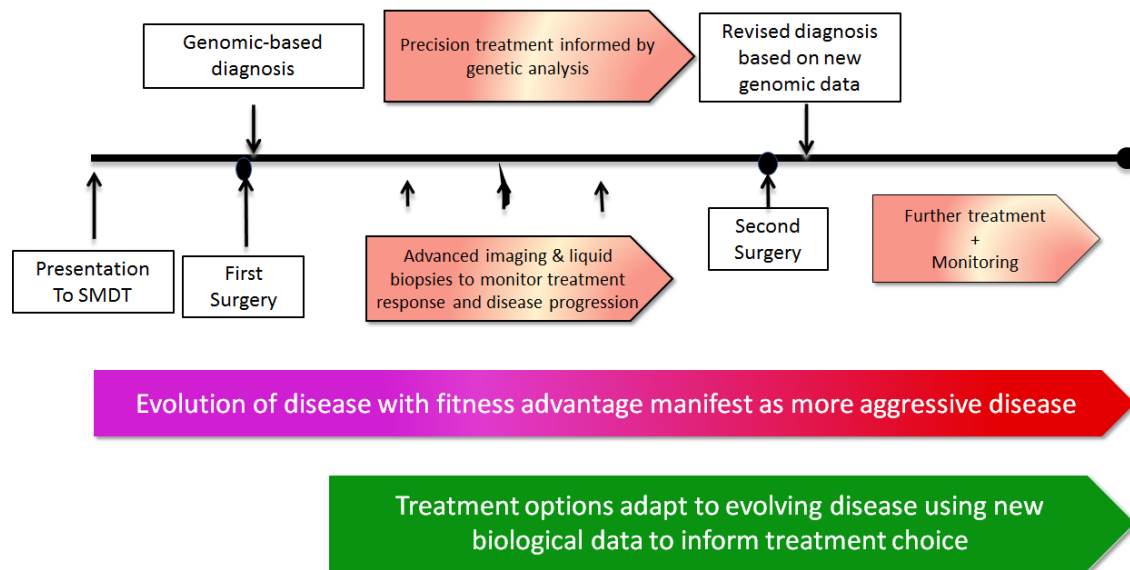
205

206

Figure 1. Usual course of glioma management. GLASS would improve the assessment of gliomas, particularly prediction of malignant transformation, treatment monitoring, and assessment of tumor alterations non-invasively with imaging and/or liquid biopsies. SMDT: Specialist multidisciplinary team; CSF: Cerebrospinal fluid; RT: Radiotherapy; TMZ: Alkylating antineoplastic agent Temozolomide; 1p19q: Short arm of chromosome 1 (1p) and long arm of chromosome 19 (19q); IDH: Isocitrate dehydrogenase; MGMT: O-6-methylguanine-DNA methyltransferase; TERT: Telomerase reverse transcriptase.

2. Molecular profiling offers new possibilities for diagnosis and therapy of gliomas

2.1 Clinical classification of adult diffuse glioma

For over a century, microscopic evaluation provided the gold standard for diagnosis of diffuse gliomas, and assessment of prognosis and therapeutic management of patients were based on histopathologic diagnosis.[24] Over the past two decades, it has become clear that combination of histopathology with specific molecular characteristics of gliomas provides a more robust and objective basis for clinical stratification.[8,11,13,17,19,25-30] Three large clinically relevant subgroups of diffuse gliomas in adults can be defined on the basis of firstly, presence or absence of mutations in the isocitrate dehydrogenase (*IDH*) 1 gene or *IDH2* gene and secondly, within the category of IDH mutated glioma, either complete 1p/19q codeletion (combined loss of the short arm of chromosome 1 and the long arm of chromosome 19) or *TP53* mutation and *ATRX* loss.[11,13,17,19,25,27-30] Most WHO grade II and III diffuse astrocytomas and oligodendrogliomas are IDH mutant, and about a third of these contain 1p/19q codeletion. In contrast, approximately 95% of glioblastomas

8

232   are IDH-wildtype.[14] IDH mutations are centered on codon 132 of *IDH1* and 172 of

233   *IDH2*. About 90% of IDH-mutant gliomas are R132H *IDH1* mutant.[31,32]   The protein

234   resulting from this mutation can be recognized by a highly specific and sensitive

235   antibody available for clinical practice[31], or through targeted sequencing. Remaining

236   IDH-mutant cases may carry alternate R132 alleles such as R132C or variants in

237   *IDH2*. Expert consensus on how these molecular data should be implemented in

238   routine clinical practice[26] led to revision in 2016 of the WHO 2007 classification of

239   CNS tumors.[8] These revisions indeed integrate histopathological and molecular data

240   [8] and for the first time, this scheme provides integrated data for diagnosis, prognostic

241   grading, and guidance of therapeutic decisions.[33,34] In the revised classification,

242   mutations in genes such as *TP53*, and *ATRX* can also be added to support or refine

243   the diagnosis but are not mandatory. However, even this greatly improved

244   classification system is predicated on primary, untreated disease; it is still unclear

245   how these molecular markers impact the biology and prognosis of post-therapy,

246   recurrent glioma. The promoter DNA methylation status of the gene *MGMT* is

247   predictive of response to temozolomide therapy in primary tumors and this status is

248   thought to be mostly stable between primary and recurrent disease [35]. The value of

249   re-testing MGMT status after disease progression is debatable and a methylated

250   MGMT promoter continues to predict treatment response at this stage.

251

2.2 Intratumoral heterogeneity in primary gliomas

253   Cancer results from a single normal cell that has acquired molecular alterations

254   providing it with a proliferative advantage. In glioma, the most frequent somatic

255   abnormalities are thought to be founding events. This included the mutations in the

256   IDH genes and mutations in the promoter of telomerase reverse transcriptase

257   (*TERT*) gene, especially characteristic of IDH-wildtype GBM and IDH-mutant,

258   1p/19q-codeleted gliomas.[25] Major aneuploidy, such as the 1p/19q co-deletion but

259   also whole chromosome 7 gain and chromosome 10 loss which is common in IDH-

260   wildtype glioma, are also thought to be glioma originating alterations. [36-38] The

261   divergence in somatic abnormality profiles between the three major glioma subtypes,

262   IDH-wildtype, IDH mutant co-deleted, IDH mutant non-codeleted, converges with

263   different patient age at diagnosis distributions, which strongly suggests that the three

264   groups represent distinct gliomagenic biologies.

265   Gliomas display significant intertumoral and intratumoral heterogeneity:

266   cancer cells from the same tumor cell of origin may contain a wide range of genetic

267   and epigenetic states.[39-41] Intratumoral heterogeneity confounds diagnosis,

268   challenges the design of effective therapies, and is a determinant of tumor

269   resistance.[42] Molecular heterogeneity in GBM has been characterized with multiple

270   approaches. For example, fluorescent in-situ hybridization (FISH) analysis of the

271   most commonly amplified receptor tyrosine kinases (RTK) in GBM (*EGFR*, *PDGFRA*

272   and *MET*) revealed a mosaic of tumor subclones marked by different RTK

273   amplifications in 2–3% of GBM,[43-45] possibly indicating cooperation between cell

274   populations. Single-cell sequencing demonstrated comparable non-overlapping

275   subclonal GBM cell populations marked by different *EGFR* truncation variants,

276   suggesting convergent evolution of *EGFR* mutations.[46] Genomic profiling of spatially

277   distinct tumor sectors has revealed partial overlap in mutation content in multiple

278   samples from IDH-mutant lower-grade glioma[19,38,47,48] and IDH-wildtype

279   GBMs.[36,37,49-51] Mutations/DNA copy number alterations in important glioma driver

280   genes such as *TP53* and *PTEN* have been found to be subclonal, i.e. not present in

281   all cells from the same tumor, suggesting they were acquired after tumor initiation.

282   These unexpected discoveries show the many options tumor cells have to

283   circumvent anti-tumorigenic hurdles such as senescence and geno7c instability. The

284   possibility of extrachromosomal oncogene amplification adds an additional layer of

285   complexity, allowing tumors to rapidly increase intratumoral heterogeneity in

286   response to a microenvironment sparse in resources.[52-57]

287   Mutation retention rates may be correlated with the geographical distance of

288   samples in the tumor,[51] and by extension, the level of heterogeneity between

289   different lesions of multifocal GBM is greater than between different areas of the

290   same GBM.[51,58,59] Spatial heterogeneity determined by genetic alterations is

291   reflected in the epigenetic patterns of different tumor sections examined by

292   combined analysis of DNA methylation and genetic abnormalities.[47,50] These

293   accumulating data suggest that intratumoral heterogeneity is encoded through a

294   genomic-epigenomic codependent relationship,[47] in which epigenetic changes may

295   modulate mutational susceptibility in proximal cells, and specific mutations dictate

296   aberrant epigenetic patterns.[47,60,61] Although gene expression signatures can be

297   used to subclassify GBMs, the predominant subtype often varies from region to

298   region within a given tumor.[37,50] This relative instability may be in part due to the

10

299   variable levels of tumor-associated non-neoplastic cells that can be found in different

300   parts of the tumor.[62,63] Single-cell RNA sequencing of GBM cells confirmed that

301   multiple subtype classifications can be detected in all tumors, often with one subtype

302   dominating the others.[51,63-65] Transcriptomics and genomics converge at the single-

303   cell level with the observation of mosaic expression of RTK, extending previous

304   observations of mosaic RTK amplification in a small subset of GBM to being a more

305   frequent disease characteristic.[64,65] Single-cell RNA sequencing further has shown

306   that all gliomas contain cellular hierarchies along an axis of undifferentiated

307   progenitors to more differentiated cell populations, reminiscent of the hematopoietic

308   stem cell hierarchy. The balances shifts towards proliferating progenitors in IDH-

309   wildtype glioma reflecting the clinically more aggressive disease course. [66-68] Such

310   developmental and functional hierarchies are associated with dynamic neural stem

311   cell expression patterns in which stem or progenitor cells may function as units of

312   evolutionary selection.[69]

313

314   **2.3 Longitudinal DNA profiling in pre-treatment and post-treatment**

315   **tumors**

316   One of the earliest reports on the effects of therapy on the tumor genomic landscape

317   analyzed a 23-patient cohort of IDH-mutant lower-grade glioma treated with

318   temozolomide chemotherapy.[70] A subset of the recurrent tumors acquired hundreds

319   of new mutations that bore a characteristic signature of temozolomide-induced

320   mutagenesis, suggesting that treatment pressure from an alkylating agent induced

321   the growth of tumor cells with new mutations (therapy-induced acquired

322   resistance).[38] These hypermutated tumors may be sensitive to immune checkpoint

323   inhibitors,[23] including programmed death-1 (PD-1) inhibitors[71] and poly-adenosine

324   diphosphate ribose polymerase (PARP) inhibitors (PARPi).[72] However, clinical trial

325   data supporting these hypotheses have yet to emerge. Another study used whole-

326   genome and multisector exome sequencing of 23 predominantly IDH-wildtype GBM

327   and matched recurrent tumors.[36] The study showed that some GBM recurrences

328   bore ancestral p53 driver mutations detectable in the primary GBM counterparts

329   (intrinsic resistance), while other recurrences were driven by branched subclonal

330   divergent mutations not present in the parental primary GBM. This may reflect

331   treatment-induced resistance through DNA mutagenesis and a distinct evolutionary

332   process.[36] As in the study of IDH-mutant lower-grade glioma, a subset of the disease

333   recurrences were characterized by an accumulation of mutations in association with

334   temozolomide treatment. Notably, this effect was limited to cases with *MGMT*

335   promoter methylation. *MGMT* is a gene in the DNA repair pathway, and mutations of

336   other pathway members, such as *MSH2* and *MSH6*, have been nominated as drivers

337   of the hypermutation process.[73] The spatiotemporal evolutionary trajectory in paired

338   gliomas between initial diagnosis and relapse was further portrayed via integrative

339   genomic and radiologic analyses. Whole-exome sequencing of 38 primary and

340   corresponding recurrent tumors revealed two prevalent patterns of tumor evolution.

341   Linear evolution, in which a recurrent tumor is genetically similar to the initial tumor,

342   was predominantly observed in a subset of recurrent tumors that relapsed adjacent

343   to the primary tumor site. Branched evolution was more common in recurrences at

344   distant sites, which were marked by a substantial genetic divergence in their

345   mutational profile from the initial tumor, with key driver alterations differing in more

346   than 30% of the cases, demonstrating branched evolution. Geographically separated

347   multifocal tumors and/or long-term recurrent tumors were seeded by distinct clones,

348   as predicted by an evolution model defined as multiverse, i.e. driven by multiple

349   subclonal cell populations.[51]

350   Comprehensive genomic analysis of the processes regulating tumor evolution

351   necessitates serial profiling of pre-treatment and post-treatment tumors. Patients

352   receiving medical care may move to a different medical center in the interval

353   between initial diagnosis and recurrence, which creates significant challenges for the

354   serial collection of tumor tissue. In an effort to elucidate the diverse evolutionary

355   dynamics by which gliomas are initiated and recur, the clonal evolution of GBM

356   under therapy was assessed from an aggregated analysis of datasets generated by

357   multiple institutions.[74] Systematic review of the exome sequences from 93 patients

358   revealed highly branched evolutionary patterns involving a Darwinian process of

359   clonal replacement in which a subset of clones with selective advantage during a

360   standard treatment regimen renders the tumor susceptible to malignant progression.

361   Mathematical modeling delineated the sequential order of somatic mutational events

362   that constitute GBM genome architecture, identifying mutations in *IDH1, PIK3CA,*

363   and *ATRX* as early events of tumor progression, whereas *PTEN, NF1, and EGFR*

364   alterations were predicted to occur at a relatively later stage of the evolution.[51]

365   Similar observations have been reported from comprehensive studies of low-grade

366 gliomas, demonstrating that the mutations in *IDH1, TP53,* and *ATRX* were frequently

367 acquired and retained throughout tumor progression from primary to relapse[19,48].

368 Additionally, these studies have demonstrated crucial insights into oncogenic

369 pathways that drive malignant progression of low-grade gliomas with mutations in

370 *IDH1,* suggesting convergence on aberrantly activated MYC, RB and RTK-RAS-

371 PI3K signaling pathways. Longitudinal profiling of paired samples continues to reveal

372 deeper insights into the genomic background of treatment-induced

373 hypermutagenesis and its potential increased aggressive clinical behavior and

374 relevance in targeted therapy and immunotherapy.[19,48,75,76] The implications of these

375 data and how these insights can be integrated into clinical practice require further

376 evaluation. Collectively, longitudinal genomic profiling will be essential in

377 implementing clinical application towards patient-tailored treatment regimen.

378

379 **2.4 Transcriptional changes during glioma progression**

380 Unsupervised transcriptome analysis of GBM converged on four expression

381 subtypes, referred to as classical, mesenchymal, neural, and proneural, which are

382 associated with specific genomic abnormalities.[14,15,18,77] The proneural and

383 mesenchymal subtypes have been most consistently confirmed in the literature,

384 whereas the neural type may simply represent GBM containing a relatively high

385 amount of admixed non-neoplastic neurons.[63,78]. Transcriptional subtyping of the

386 relatively homogeneous IDH-mutant and 1p/19q-codeleted groups have been less

387 emphasized in the literature, as these cases usually carry a proneural signature.[12,14]

388 While expression subtype classification is a widely used research tool, it has not

389 been shown to correlate with clinical outcome, and has not been incorporated in the

390 recent WHO CNS tumor classification update. Much is still unknown about the

391 drivers of transcriptional subclasses in GBM, their plasticity and how they evolve

392 under therapy. A switch from proneural to mesenchymal expression has been

393 observed upon disease recurrence and proposed as a source of treatment

394 resistance in GBM relapse,[18,79-81] but the relevance of this phenomenon in glioma

395 progression remains ambiguous, particularly considering a. the increased fraction of

396 microglial/macrophage cells in mesenchymal GBM that confound subtype

397 characterization[62,63] and b. glioma neurospheres derived from mesenchymal GBM

398 are frequently classified as proneural.[79] Deriving an expression subtype classification

399   on the basis of glioma-intrinsic genes has maintained the proneural, classical, and

400   mesenchymal classes [63]. Determining subtypes in a cohort of 91 IDH-wildtype GBM

401   showed subtype switching following therapy and disease relapse in 45% of the

402   cohort [63]. These patterns converged with changes in the tumor microenvironment,

403   corroborating observations from single-cell transcriptomics that every GBM

404   comprises different subtype mixtures but also revealing that *NF1* loss results in

405   macrophage/microglia recruitment. The ability of genomic abnormalities to regulate

406   the tumor microenvironment suggest cooperation and shows how tumors act as a

407   system or an organ, rather than an aggregation of individual aberrant cells.

408

2.5 Epigenetic changes during glioma progression

410   DNA methylation profiling of gliomas has prognostic value independent of the age of

411   the patient and the tumors pathologic grade.[11] Although clonal selection under

412   therapy of genetic mutations nominates mutations as drivers of therapy resistance,

413   strong evidence also suggests that evolutionary selection acting on the epigenome,

414   in the absence of genetic changes, affords plasticity of cells to resist therapy.[11,47] For

415   example, recurrent IDH-mutant gliomas profiled for mutations and DNA methylation

416   independently evolved deregulation of their cell cycle programs, through genetic

417   mutations or epigenetic mechanisms.[47] Epigenetic convergence on genetically

418   deregulated biological processes demonstrates that epigenetic abnormalities provide

419   cell subpopulations with fitness advantages that could undermine therapy.

420   Nearly all IDH-mutant gliomas exhibit a characteristic CpG island

421   hypermethylator phenotype (G-CIMP).[12] Possible hypotheses for this relationship

422   are as follows (i) DNA hypermethylation induces silencing of key extracellular matrix

423   and cell migration gene promoters,[12] (ii) DNA methylation mediates alteration of

424   chromosome topography, leading to oncogene upregulation[82,83] (iii) histone

425   methylation-related changes in gene expression, (iv) DNA hypermethylation

426   associated with mutant IDH may play a role in creating an immunosuppressed

427   microenvironment.[84]

428   G-CIMP tumors can be further parsed into subsets with reduced genome-wide

429   DNA methylation levels.[11] While almost all IDH-mutant tumors are G-CIMP at

430   diagnosis, a longitudinal analysis showed that 34% of cases exhibited demethylation

431   towards G-CIMP-intermediate or G-CIMP-low DNA methylation at recurrence.[85]

432   Substantial epigenetic heterogeneity between tumor samples from the same patient

433   collected at subsequent surgeries was also observed in a cohort of 112 primary

434   GBM patients [86]. Characteristic trends in DNA methylation between primary and

435   relapsed GBM included a prominent demethylation of Wnt signaling gene promoters,

436   which was associated with worse patient outcome.   Moreover, patients whose

437   primary tumors harbored higher levels of DNA methylation erosion showed longer

438   progression-free survival and a trend towards longer overall survival [86]. This study

439   also explored associations between changes in DNA methylation and magnetic

440   resonance imaging (MRI) and digital pathology data, highlighting the connectedness

441   of the various levels of molecular, cellular and phenotypic heterogeneity in GBM.

442   Analysis of larger cohorts is needed to determine the association between genomic

443   and epigenetic deregulation.

444

445   **2.6 Imaging and (epi)genomics**

446   MRI is a crucial part of standard diagnostic work-up and follow-up of brain tumor

447   patients. It is noninvasive, and owing to the lack of radiation exposure, repeat

448   imaging is not harmful. Conventional MR tumor imaging includes precontrast and

449   post-contrast T1-weighted (T1w) and T2-weighted (T2w)/T2w fluid-attenuated

450   inversion recovery (T2-FLAIR) imaging to assess tumor location, size, and certain

451   macrostructural features.[87] Newer techniques such as perfusion imaging provide a

452   measure of tumor vascularization in terms of relative cerebral blood volume, which

453   correlates with tumor grade.[88,89] Relative cerebral blood volume reflects biological

454   behavior of tumors, which might relate to molecular profiles.

455   In the rapidly growing field of research called radiogenomics,[90] a rich set of

456   quantitative imaging features are linked with genomic profiles. It has recently been

457   applied in the context of high-grade glioma.[90-92] Given the major differences in DNA

458   characteristics, gene expression profiles, and DNA methylation profiles, a priority of

459   radiogenomics research on glioblastoma is to identify MR imaging based biomarkers

460   of molecularly defined lower-grade glioma subtypes such as IDH-mutant versus

461   wildtype and 1p/19q codeleted versus non-codeleted. Noninvasive phenotypical

462   assessment has several clear advantages. First, it provides an early test to stratify

463   IDH-mutant, 1p/19q non-codeleted glioma tumors, identifying those patients who are

464   candidates for the most aggressive therapeutical strategies and those for whom a

15

465   more conservative approach may be preferred. Providing reliable prognostic

466   information through MR imaging can improve patients' quality of life by postponing,

467   and potentially obviating the need for surgery for a subset of patients.[93] Second, it

468   would be a means of selecting and tracking patients for personalized treatment

469   regimens in clinical trials.[94] Third, a detailed global assessment of spatial and

470   longitudinal heterogeneity of gliomas becomes feasible.[95]

471

3. Barriers to progress

473   The major obstacle to glioma patients currently is a lack of effective treatments, yet

474   we have little understanding of why treatments fail. These failures are likely related

475   to dynamic tumor evolution where treatment-resistant glioma cells are favored over

476   treatment-sensitive cells. As a result, therapy has profound effects on tumor

477   composition by activating intrinsic and adaptive resistance mechanisms, most clearly

478   reflected by the temozolomide induced hypermutator phenotype [70]. Such processes

479   may be directly induced by the therapy itself, or they are the result of survival and

480   clonal expansion of tumor cells with genetic, epigenetic and/or regulatory alterations

481   that confer drug resistance. Moreover, tumor cells may attenuate the immune

482   response, locally and systemically, to prevent immunological recognition and

483   clearance. All of these processes result in molecular characteristics of the recurrent

484   tumor that differ in significant ways from those found in the primary tumor [36,38].

485   To improve the outcomes of patients with gliomas, we need to explore new

486   therapeutic approaches based on a thorough understanding of treatment-induced

487   molecular and genetic diversity that leads to resistance. The TCGA glioma effort and

488   similar initiatives elsewhere have established comprehensive portraits of the

489   interpatient variability of untreated glioma genomes. Single cell sequencing and

490   barcoding experiments have demonstrated functional hierarchies providing important

491   insights into characteristics of the most relevant cells to target [66-68]. We are

492   increasingly able to infer the life history of glioma, from tumor-initiating events such

493   as *IDH1* mutation to tumor-promoting events such as RTK alterations. A detailed

494   understanding of the biological diversity within every tumor following clinical

495   presentation and disease progression is needed if we are to successfully understand

496   how treatment affects glioma progression, a needed step towards integration of

497   precision therapeutics into clinical decision making. These considerations also

498   highlight the danger in considering treatment options for patients with recurrent

499   tumors solely on the basis of the molecular analysis of their treatment-naïve tumors.

500   This is particularly important in the setting of clinical research, which often recruits

501   patients with recurrent GBM to evaluate drugs developed on the basis of mechanistic

502   data obtained on treatment-naïve tumors.

503   Studying heterogeneity and spatiotemporal evolution of cancer in general, and

504   particularly in brain cancer, is challenging. Many tumor samples, and therefore large-

505   scale collaboration, are needed to achieve meaningful comprehensive results. For

506   example, to identify 80% of all somatic alterations occurring in at least 3% of the

507   patient population, a cohort of 500 samples would be needed.[96] It is crucial to recruit

508   sufficient numbers to validate findings and to capture low-frequency alterations.

509   Individual research groups typically do not have the resources to use a multiplatform

510   analysis of their samples, owing to cost or availability of expertise. Existing

511   longitudinal datasets that have been published consist of a mixture of different

512   modalities, ranging from only exomes[38] or DNA methylation profiles[47,86] to a

513   combination of exome sequencing, RNA sequencing, and DNA copy number

514   profiling,[36,63] thwarting meta-analyses based on cross-publication comparisons. The

515   value of establishing a comprehensive multiplatform reference dataset quickly has

516   been demonstrated by the success of TCGA glioma projects, which have led to a

517   fundamental reclassification of gliomas by the WHO[8] and are highly cited.[9,12-14]

518   Similarly, a consortium would be the most effective approach assembling the large

519   cohorts of primary and recurrent pairs needed to identify somatic alterations enriched

520   after disease progression. Systematizing and standardizing what we do and how we

521   do it will be essential for affecting paradigmatic change to clinical practice in neuro-

522   oncology. This philosophy is at the core of the international GLASS consortium.

523

524   4. The Glioma Longitudinal AnalySiS (GLASS) consortium

525   The rapidly growing body of knowledge in molecular and genomic data is refining

526   clinical diagnosis and prognostication (exemplified by the revised fourth edition of the

527   WHO classification of CNS tumors published in 2016[8]), but has not resulted in

528   improvements clinical outcomes, particularly in the more aggressive gliomas. This is

529   evidenced by numerous trials that have failed to reach their primary endpoint.[97,3,98]

530   These successes and failures highlight the need for large-scale collaborations that to

531 help us understand the impact of treatment on evolutionary dynamics and, most

532 importantly, why treatments fails?

533   The recognition of the need is why we initiated the GLASS consortium: to

534 achieve power from the use of large numbers of paired samples from all contributors.

535 GLASS aims to perform comprehensive molecular profiling of matched primary and

536 recurrent glioma specimens from an unprecedented 1500 patients, 500 in each of

537 the three major glioma molecular subtypes: 1. IDH-wildtype; 2. IDH-mutant; and 3.

538 IDHmutant with 1p/19q codeletion. The consortium at the time of writing includes

539 investigators from 32 academic hospitals, universities, and research institutes from

540 12 countries, (see list of participants on the GLASS website, http://www.glass-

541 consortium.org). By analogy with the International Cancer Genomics Consortium [99]

542 GLASS is structured into country-specific franchises (GLASS-NL, GLASS-AT,

543 GLASS-AU, GLASS-Korea, etc.) led by local investigators who are invested in the

544 team's overall goal of assembling a meaningful sample cohort of pretreatment and

545 post-treatment samples for each glioma grade and type, while taking advantage of

546 country-specific opportunities. This enables each GLASS branch to have unique

547 features that allow deeper analysis of subcohorts, that is, with additional imaging

548 annotation, parallel characterization of drug response through xenografting of tumor

549 samples, specific focus on a glioma subtype, etc., thereby making them competitive

550 and enabling them to address non-overlapping aspects of the phenotypic diversity

551 seen in the clinic. Country-specific branches will be coordinated to connect with the

552 larger analyses, to drive specific research topics for both. There are no explicit

553 restrictions on publishing, that is, each group is invited to publish their substudies

554 independently. The overall goal is to establish a reference data set by pooling

555 samples and aggregate data from all multiplatform analyses, countries and

556 substudies, and to make datasets comparable through coordinated sample and data

557 processing guidelines. Country franchises are centrally connected through a number

558 of committees, each overseeing different aspects of the analysis (Figure 2).

559

18

Figure 2. Overview of GLASS committees. Details on committee mandates are provided in section 4.

560

4.1 Biospecimens and characterization platforms

562 Biospecimens from gliomas are often snap frozen or conserved formalin fixed,

563 paraffin embedded (FFPE). For genomic and transcriptomic analyses, snap frozen

564 material is preferred, while historically FFPE is the common approach to tissue

565 preservation. Methods for generating sequencing data from FFPE material are

566 increasingly improving, with 5–20% of samples failing quality controls. Given that

567 samples from multiple timepoints are required for inclusion into GLASS, patients for

568 whom only FFPE material is available are twice as likely to not yield sufficient high

569 quality DNA. RNA extracted from glioma tissue is often highly degraded resulting in

570 higher attrition rates[100], but high quality RNA sequencing data from FFPE samples

571 has been reported [101]. For DNA methylation profiling of FFPE material, a recent

572 study focusing on primary glioblastoma reported a high success rate using the

573 reduced representation bisulfite sequencing assay [86]. While we require the

574 availability of a matching germline sample (often but not always from blood) for

575 inclusion of DNA sequencing data into GLASS, cases without a germline match may

576 be candidates for transcriptome and DNA methylation analysis. Ideally, we aim to

577 generate DNA, RNA and epigenomic sequencing data from every patient. Single-cell

578   analysis methods require fresh tissue from which individual cells can be dissociated;

579   they are currently outside the scope of GLASS, but may be considered in the future

580   as the project evolves or as part of specific subprojects. Similarly subsets of the

581   GLASS cohort will be compared longitudinally by spatial correlation using multisector

582   analysis (3–6 samples per tumor) to understand whether any differences between

583   paired tumor samples are the result of intratumoral heterogeneity or longitudinal

584   heterogeneity. Where available, these will be correlated with conventional and novel

585   MR imaging to explore spatiotemporal heterogeneity noninvasively. We aim to take

586   current radiogenomic approaches further, not only to establish features of genetic

587   characteristics at first diagnosis, but also in relation to molecular alterations over time

588   (including under pressure of standard therapy).

589   Comprehensive genomic sequencing is needed to identify patterns of disease

590   evolution as well as key mutations and chromosomal alterations that confer

591   resistance to standard radiation, temozolomide, and novel clinical trial therapies.

592   Sequencing paradigms and their costs are rapidly evolving, and each method

593   provides different but often complementary information. There is no consensus on

594   optimal methods. With the accessibility of $1000 per biospecimen whole genome

595   sequencing (WGS), the costs of WGS and whole exome sequencing (WES) have

596   become comparable. WES has better sensitivity in detecting mutations in coding

597   regions, but does not interrogate noncoding regions of the genome, structural

598   variants, or noncoding copy number variants. The comprehensive nature of WGS

599   enables analysis of evolution and clonality at higher resolution. WGS and WES

600   combined may provide the optimal window on the breadth, depth, and allelic fraction

601   of somatic events. However, where limitations in tissue or resources mandate a

602   choice of one or the other, the decision will depend on the purpose of the (sub)

603   project.

604

605   **4.2 GLASS committees**

606   GLASS has established different committees with the expertise to coordinate the

607   various aspects of the consortium. They include pathology, clinical annotation, data

608   infrastructure, ethics and publication, and funding committees.

609   **4.2.1 The GLASS pathology committee** maintains centralized classification and

610   tissue processing. The committee has set up a panel of specific inclusion criteria for

611   tissue samples. A prerequisite for inclusion is that the patient has given informed

612   consent to donate tissue to research and that an adequate blood sample or any

613   other adequate source of genomic DNA is available. Both FFPE and snap-frozen

614   tissue samples will be included in the GLASS study and evaluated by the committee.

615   The area of viable tumor tissue should be at least 50 mm^2 and the tumor cell

616   percentage should be higher than 50%. Hematoxylin and eosin slides of each

617   sample will be digitized by an automated slide scanner and the images will be stored

618   on a central server in order to be accessible by all members of the GLASS expert

619   pathology committee. An anonymized pathology report of both the original and the

620   recurrent tumor has to be submitted for review to the committee with information on

621   microscopic (including immunohistochemical) findings and, if performed, results of

622   molecular analyses, as well as the integrated diagnosis. On the basis of this

623   information, the GLASS expert pathology committee will formulate a (tentative)

624   review diagnosis and thereby select patient samples that can be used for further

625   study by the GLASS consortium. Corresponding whole-slide images of all patient

626   samples that are included in GLASS will then be made available as a digital resource

627   for further image analysis [102,103].

628   **4.2.2 The GLASS clinical annotation committee** maintains standardized data

629   processing, data management and data sharing. The currently available large-scale

630   datasets suffer from relatively weak clinical annotation. Consequently, linkage of

631   genotype with clinical and morphological phenotype remains to be fully exploited in

632   primary and recurrent settings. The GLASS clinical annotation committee will

633   address this by standardizing clinical and imaging data collection for prospective

634   studies and oversee aggregation of the clinical and imaging data from patients

635   whose profiles are already included in the composite dataset. Insight into

636   mechanisms of response and resistance and exposed therapeutic vulnerabilities will

637   be fed into current and future clinical trial designs by GLASS investigators and trials

638   designed collaboratively in academia and industry or across both. This strategy will

639   require an integrated bioinformatics interface across the molecular and clinical

640   research. The necessary data processing infrastructure will be developed by the

641   GLASS consortium and distributed among its franchises, to ensure compatibility,

642   comparability, and reproducibility. Each individual franchise will make clinical,

643   imaging, and molecular data accessible in a comprehensible way by integrating

644   clinical, imaging, and molecular parameters to explore correlation with relapse data.

645   Currently, radiology and imaging are part of the clinical annotation committee. By

646   mapping imaging features in a voxel-wise manner and correlating these spatially with

647   molecular alterations obtained from different parts of the tumor we aim to assess the

648   entire tumor and to determine intratumoral heterogeneity.

649   **4.2.3 The GLASS data infrastructure committee** maintains standardized data

650   processing, data management and data sharing. A characteristic of the GLASS

651   consortium is that data will be generated at multiple institutions distributed over

652   multiple countries. As the regulations pertaining to ethical use of sequencing

653   datasets are continuously evolving, GLASS will follow the example set by ICGC to

654   perform decentralized data analysis to avoid cross-border exchange of patient-

655   sensitive raw sequencing data. The GLASS data infrastructure committee has

656   developed Docker software images that can be shared by participating institutions to

657   ensure analysis uniformity. Like a shipping container, a Docker image packages one

658   or more software tools to establish a workflow resembling an executable application.

659   Comparable to platform-independent Java software, the ready-to-run Docker images

660   are independent of the local computational environment. The GLASS participants

661   run the Docker image locally, which initializes a per-sample-per-analysis Docker

662   container, resulting in data analysis using an identical software environment and run

663   parameters. Docker images are available to process exome sequencing data, which

664   includes alignment, quality control, mutation calling, and DNA copy number

665   estimation. Comparable Docker images are ready for processing of whole-genome

666   sequencing and transcriptome sequencing data. Docker images are available for

667   download through http://docker.glass-consortium.org *(RV: currently pending)*.

668   The data infrastructure committee will also coordinate mechanisms for

669   dissemination of results, as to widely share datasets with the community. We may

670   explore mechanisms such as the Genomic Data Commons, or similar, in order to

671   align our efforts with other molecular profiling studies.

672   **4.2.4 The GLASS ethics and publications committee** was created to identify and

673   address critical ethical, legal and social questions faced by researchers and patients

674   participating in the GLASS program. The guidelines established by this group will

675   continue to inform future policies that ensure effective and fair use of cancer

676   genomic information coupled with relevant clinical annotations. All participating sites

677   in the program have institutional ethics approval for data protection and the use of

678   tissue and/or DNA and clinical and where applicable imaging data from patients that

22

679 have given written informed consent. Data will be made available rapidly after

680 generation for community research use. Publication guidelines will follow that

681 established by the TCGA policy for authorship and publication.

682 **5. Final remarks and perspectives**

683 Survival and quality of life for patients with diffuse gliomas remains dismal with

684 standard treatments. Diffuse glioma is a fatal disease with an enormous societal

685 burden as a result of short survival following high-grade disease and the relatively

686 young age at diagnosis of lower-grade disease. This not only affects patients in the

687 prime of their life, but also puts enormous burden on their immediate entourage, as

688 they need extensive supportive care and navigation through a complicated medical

689 landscape, and difficulties with medical costs and insurance. While cures of diffuse

690 gliomas remain elusive, our patients demand better therapies and, with no

691 substantive impact of molecular medicine, in practice treatments remain a 'one size

692 fits all'. The GLASS Consortium will enable the improvement of clinical outcomes by

693 establishing a broadly useful reference data set that will provide pivotal new insights

694 into mechanisms used by gliomas to defy therapeutic challenges. For example,

695 hypermutation following temozolomide treatment occurs in up to 15% of glioma, but

696 too few samples have been profiled to understand what is driving this process or to

697 identify biomarkers predictive of a TMZ associated hypermutator phenotype. GLASS

698 will have the power to identify molecular markers indicating evolution from newly

699 diagnosed to highly aggressive therapy-resistant malignancy, in addition to

700 molecular aberrations that occur under pressure from different therapeutic

701 modalities. It will also allow for the identification of currently undiscovered molecular

702 targets for resistance-prevention agents that might be co-administered with classical

703 therapies. Systematic correlation of the molecular information with clinical, imaging,

704 and pathology data will help improve interpretation of prognostic findings in the

705 course of the disease.

706   Finally, and importantly, GLASS is an opportunity for interchange of

707 knowledge among an international group of collaborators to ultimately build smarter

708 clinical trials and develop therapies that will extend survival and improve the quality

709 of life of people with diffuse gliomas. GLASS is well positioned to demonstrate the

710 value of well-coordinated collaborative efforts. To that extent, new investigators are

711 invited to join to the Consortium, where major criteria for participation are the ability

712   to offer datasets of longitudinally profiled glioma patients or the availability of suitable

713   tissue samples.

714   In summary, we hope that through the GLASS Consortium, we continue the

715   immeasurable success of The Cancer Genome Atlas while increasing the focus on

716   making a difference to patients and their families. Therapy resistance is what kills

717   patients, and GLASS will inform on how to avoid it.

718

Acknowledgements

736

Conflicts of interest

738

References:

739

740 **1.** Ostrom QT, Gittleman H, Xu J, et al. CBTRUS Statistical Report: Primary Brain and
741 Other Central Nervous System Tumors Diagnosed in the United States in 2009-
742 2013. *Neuro Oncol.* 2016; 18(suppl_5):v1-v75.

743 **2.** Khasraw M, Lassman AB. Advances in the treatment of malignant gliomas. *Curr*
744 *Oncol Rep.* 2010; 12(1):26-33.

745 **3.** Khasraw M, Ameratunga MS, Grant R, Wheeler H, Pavlakis N. Antiangiogenic
746 therapy for high‐grade glioma. *The Cochrane Library.* 2014.

747 **4.** Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical
748 review. *Jama.* 2013; 310(17):1842-1850.

749 **5.** Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ. Exciting
750 new advances in neuro-oncology: the avenue to a cure for malignant glioma. *CA*
751 *Cancer J Clin.* 2010; 60(3):166-193.

752 **6.** Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and
753 adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352(10):987-996.

754 **7.** Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria
755 for high-grade gliomas: response assessment in neuro-oncology working group. *J*
756 *Clin Oncol.* 2010; 28(11):1963-1972.

757 **8.** Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization
758 Classification of Tumors of the Central Nervous System: a summary. *Acta*
759 *Neuropathol.* 2016; 131(6):803-820.

760 **9.** Cancer Genome Atlas Research N. Comprehensive genomic characterization
761 defines human glioblastoma genes and core pathways. *Nature.* 2008;
762 455(7216):1061-1068.

763 **10.** Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of
764 glioblastoma. *Cell.* 2013; 155(2):462-477.

765 **11.** Ceccarelli M, Barthel FP, Malta TM, et al. Molecular Profiling Reveals Biologically
766 Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell.* 2016;
767 164(3):550-563.

768 **12.** Noushmehr H, Weisenberger DJ, Diefes K, et al. Identification of a CpG island
769 methylator phenotype that defines a distinct subgroup of glioma. *Cancer cell.*
770 2010; 17(5):510-522.

771 **13.** TCGA_Network, Brat DJ, Verhaak RG, et al. Comprehensive, Integrative Genomic
772 Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med.* 2015; 372(26):2481-
773 2498.

774 **14.** Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies
775 clinically relevant subtypes of glioblastoma characterized by abnormalities in
776 PDGFRA, IDH1, EGFR, and NF1. *Cancer cell.* 2010; 17(1):98-110.

777 **15.** Gravendeel LA, Kouwenhoven MC, Gevaert O, et al. Intrinsic gene expression
778 profiles of gliomas are a better predictor of survival than histology. *Cancer Res.*
779 2009; 69(23):9065-9072.

780 **16.** Bettegowda C, Agrawal N, Jiao Y, et al. Mutations in CIC and FUBP1 contribute to
781 human oligodendroglioma. *Science.* 2011; 333(6048):1453-1455.

782 **17.** Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J*
783 *Med.* 2009; 360(8):765-773.

784 **18.** Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade
785 glioma predict prognosis, delineate a pattern of disease progression, and
786 resemble stages in neurogenesis. *Cancer Cell.* 2006; 9(3):157-173.

787   **19.**   Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in
788   grade II and III gliomas. *Nat Genet.* 2015; 47(5):458-468.
789   **20.**   Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define
790   distinct epigenetic and biological subgroups of glioblastoma. *Cancer cell.* 2012;
791   22(4):425-437.
792   **21.**   Eskilsson E, Verhaak RG. Longitudinal genomic characterization of brain tumors
793   for identification of therapeutic vulnerabilities. *Neuro Oncol.* 2016; 18(8):1037-
794   1039.
795   **22.**   Blank CU, Haanen JB, Ribas A, Schumacher TN. CANCER IMMUNOLOGY. The
796   "cancer immunogram". *Science.* 2016; 352(6286):658-660.
797   **23.**   Osuka S, Van Meir EG. Overcoming therapeutic resistance in glioblastoma: the
798   way forward. *J Clin Invest.* 2017; 127(2):415-426.
799   **24.**   Perry A, Wesseling P. Histologic classification of gliomas. *Handb Clin Neurol.*
800   2016; 134:71-95.
801   **25.**   Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma Groups Based on
802   1p/19q, IDH, and TERT Promoter Mutations in Tumors. *N Engl J Med.* 2015;
803   372(26):2499-2508.
804   **26.**   Louis DN, Perry A, Burger P, et al. International Society Of Neuropathology--
805   Haarlem consensus guidelines for nervous system tumor classification and
806   grading. *Brain Pathol.* 2014; 24(5):429-435.
807   **27.**   Dubbink HJ, Atmodimedjo PN, Kros JM, et al. Molecular classification of
808   anaplastic oligodendroglioma using next-generation sequencing: a report of the
809   prospective randomized EORTC Brain Tumor Group 26951 phase III trial. *Neuro
810   Oncol.* 2016; 18(3):388-400.
811   **28.**   Yan W, Zhang W, You G, et al. Molecular classification of gliomas based on whole
812   genome gene expression: a systematic report of 225 samples from the Chinese
813   Glioma Cooperative Group. *Neuro Oncol.* 2012; 14(12):1432-1440.
814   **29.**   Jiao Y, Killela PJ, Reitman ZJ, et al. Frequent ATRX, CIC, FUBP1 and IDH1
815   mutations refine the classification of malignant gliomas. *Oncotarget.* 2012;
816   3(7):709-722.
817   **30.**   Wiestler B, Capper D, Sill M, et al. Integrated DNA methylation and copy-number
818   profiling identify three clinically and biologically relevant groups of anaplastic
819   glioma. *Acta Neuropathol.* 2014; 128(4):561-571.
820   **31.**   Capper D, Weissert S, Balss J, et al. Characterization of R132H mutation-specific
821   IDH1 antibody binding in brain tumors. *Brain Pathol.* 2010; 20(1):245-254.
822   **32.**   Horbinski C. What do we know about IDH1/2 mutations so far, and how do we
823   use it? *Acta Neuropathol.* 2013; 125(5):621-636.
824   **33.**   Olar A, Wani KM, Alfaro-Munoz KD, et al. IDH mutation status and role of WHO
825   grade and mitotic index in overall survival in grade II-III diffuse gliomas. *Acta
826   Neuropathol.* 2015; 129(4):585-596.
827   **34.**   Reuss DE, Sahm F, Schrimpf D, et al. ATRX and IDH1-R132H
828   immunohistochemistry with subsequent copy number analysis and IDH
829   sequencing as a basis for an "integrated" diagnostic approach for adult
830   astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol.* 2015;
831   129(1):133-146.
832   **35.**   Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of
833   recurrent glioblastoma--are we there yet? *Neuro Oncol.* 2013; 15(1):4-27.

834   **36.**   Kim H, Zheng S, Amini SS, et al. Whole-genome and multisector exome
835   sequencing of primary and post-treatment glioblastoma reveals patterns of
836   tumor evolution. *Genome Res.* 2015; 25(3):316-327.
837   **37.**   Sottoriva A, Spiteri I, Piccirillo SG, et al. Intratumor heterogeneity in human
838   glioblastoma reflects cancer evolutionary dynamics. *Proceedings of the National*
839   *Academy of Sciences of the United States of America.* 2013; 110(10):4009-4014.
840   **38.**   Johnson BE, Mazor T, Hong C, et al. Mutational analysis reveals the origin and
841   therapy-driven evolution of recurrent glioma. *Science.* 2014; 343(6167):189-
842   193.
843   **39.**   Navin N, Kendall J, Troge J, et al. Tumour evolution inferred by single-cell
844   sequencing. *Nature.* 2011; 472(7341):90-94.
845   **40.**   Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched
846   evolution revealed by multiregion sequencing. *N Engl J Med.* 2012; 366(10):883-
847   892.
848   **41.**   Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C. Defining the mode of
849   tumour growth by clonal analysis. *Nature.* 2012; 488(7412):527-530.
850   **42.**   Bedard PL, Hansen AR, Ratain MJ, Siu LL. Tumour heterogeneity in the clinic.
851   *Nature.* 2013; 501(7467):355-364.
852   **43.**   Snuderl M, Fazlollahi L, Le LP, et al. Mosaic amplification of multiple receptor
853   tyrosine kinase genes in glioblastoma. *Cancer cell.* 2011; 20(6):810-817.
854   **44.**   Little SE, Popov S, Jury A, et al. Receptor tyrosine kinase genes amplified in
855   glioblastoma exhibit a mutual exclusivity in variable proportions reflective of
856   individual tumor heterogeneity. *Cancer Res.* 2012; 72(7):1614-1620.
857   **45.**   Szerlip NJ, Pedraza A, Chakravarty D, et al. Intratumoral heterogeneity of
858   receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma
859   defines subpopulations with distinct growth factor response. *Proceedings of the*
860   *National Academy of Sciences of the United States of America.* 2012; 109(8):3041-
861   3046.
862   **46.**   Francis JM, Zhang CZ, Maire CL, et al. EGFR variant heterogeneity in glioblastoma
863   resolved through single-nucleus sequencing. *Cancer Discov.* 2014; 4(8):956-971.
864   **47.**   Mazor T, Pankov A, Johnson BE, et al. DNA Methylation and Somatic Mutations
865   Converge on the Cell Cycle and Define Similar Evolutionary Histories in Brain
866   Tumors. *Cancer cell.* 2015; 28(3):307-317.
867   **48.**   Bai H, Harmanci AS, Erson-Omay EZ, et al. Integrated genomic characterization of
868   IDH1-mutant glioma malignant progression. *Nat Genet.* 2016; 48(1):59-66.
869   **49.**   Kumar A, Boyle EA, Tokita M, et al. Deep sequencing of multiple regions of glial
870   tumors reveals spatial heterogeneity for mutations in clinically relevant genes.
871   *Genome Biol.* 2014; 15(12):530.
872   **50.**   Aubry M, de Tayrac M, Etcheverry A, et al. From the core to beyond the margin: a
873   genomic picture of glioblastoma intratumor heterogeneity. *Oncotarget.* 2015;
874   6(14):12094-12109.
875   **51.**   Lee JK, Wang J, Sa JK, et al. Spatiotemporal genomic architecture informs
876   precision oncology in glioblastoma. *Nat Genet.* 2017.
877   **52.**   deCarvalho AC, Kim H, Poisson LM, et al. Extrachromosal DNA elements can drive
878   disease evolution in glioblastoma. *bioRxiv.* 2016.
879   **53.**   Nathanson DA, Gini B, Mottahedeh J, et al. Targeted therapy resistance mediated
880   by dynamic regulation of extrachromosomal mutant EGFR DNA. *Science.* 2014;
881   343(6166):72-76.

882 54. Turner KM, Deshpande V, Beyter D, et al. Extrachromosomal oncogene
883   amplification drives tumour evolution and genetic heterogeneity. *Nature.* 2017.
884 55. Nikolaev S, Santoni F, Garieri M, et al. Extrachromosomal driver mutations in
885   glioblastoma and low-grade glioma. *Nat Commun.* 2014; 5:5690.
886 56. Vogt N, Lefevre SH, Apiou F, et al. Molecular structure of double-minute
887   chromosomes bearing amplified copies of the epidermal growth factor receptor
888   gene in gliomas. *Proceedings of the National Academy of Sciences of the United*
889   *States of America.* 2004; 101(31):11368-11373.
890 57. Zheng S, Fu J, Vegesna R, et al. A survey of intragenic breakpoints in glioblastoma
891   identifies a distinct subset associated with poor survival. *Genes Dev.* 2013;
892   27(13):1462-1472.
893 58. Abou-El-Ardat K, Seifert M, Becker K, et al. Comprehensive molecular
894   characterization of multifocal glioblastoma proves its monoclonal origin and
895   reveals novel insights into clonal evolution and heterogeneity of glioblastomas.
896   *Neuro Oncol.* 2017; 19(4):546-557.
897 59. Liu Q, Liu Y, Li W, et al. Genetic, epigenetic, and molecular landscapes of
898   multifocal and multicentric glioblastoma. *Acta Neuropathol.* 2015; 130(4):587-
899   597.
900 60. Raiber E-A, Beraldi D, Martínez Cuesta S, et al. Base resolution maps reveal the
901   importance of 5-hydroxymethylcytosine in a human glioblastoma. *npj Genomic*
902   *Medicine.* 2017; 2(1):6.
903 61. Johnson KC, Houseman EA, King JE, von Herrmann KM, Fadul CE, Christensen BC.
904   5-Hydroxymethylcytosine localizes to enhancer elements and is associated with
905   survival in glioblastoma patients. *Nat Commun.* 2016; 7:13177.
906 62. Engler JR, Robinson AE, Smirnov I, et al. Increased microglia/macrophage gene
907   expression in a subset of adult and pediatric astrocytomas. *PLoS One.* 2012;
908   7(8):e43339.
909 63. Wang Q, Hu B, Hu X, et al. Tumor Evolution of Glioma-Intrinsic Gene Expression
910   Subtypes Associates with Immunological Changes in the Microenvironment.
911   *Cancer cell.* 2017; 32(1):42-56 e46.
912 64. Muller S, Liu SJ, Di Lullo E, et al. Single-cell sequencing maps gene expression to
913   mutational phylogenies in PDGF- and EGF-driven gliomas. *Mol Syst Biol.* 2016;
914   12(11):889.
915 65. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral
916   heterogeneity in primary glioblastoma. *Science.* 2014; 344(6190):1396-1401.
917 66. Tirosh I, Venteicher AS, Hebert C, et al. Single-cell RNA-seq supports a
918   developmental hierarchy in human oligodendroglioma. *Nature.* 2016;
919   539(7628):309-313.
920 67. Venteicher AS, Tirosh I, Hebert C, et al. Decoupling genetics, lineages, and
921   microenvironment in IDH-mutant gliomas by single-cell RNA-seq. *Science.* 2017;
922   355(6332).
923 68. Lan X, Jorg DJ, Cavalli FMG, et al. Fate mapping of human glioblastoma reveals an
924   invariant stem cell hierarchy. *Nature.* 2017; 549(7671):227-232.
925 69. Greaves M, Maley CC. Clonal evolution in cancer. *Nature.* 2012; 481(7381):306-
926   313.
927 70. Hunter C, Smith R, Cahill DP, et al. A hypermutation phenotype and somatic
928   MSH6 mutations in recurrent human malignant gliomas after alkylator
929   chemotherapy. *Cancer Res.* 2006; 66(8):3987-3991.

930   **71.**   Bouffet E, Larouche V, Campbell BB, et al. Immune Checkpoint Inhibition for
931   Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic
932   Mismatch Repair Deficiency. *Journal of Clinical Oncology.* 2016; 34(19):2206-
933   2211.
934   **72.**   McDonald KL, Khasraw M, Jue TR, Joshi S, Yin JL. Genomically unstable
935   glioblastoma are sensitive to Parp inhibition. *Cancer Research.* 2015; 75.
936   **73.**   Yip S, Miao J, Cahill DP, et al. MSH6 mutations arise in glioblastomas during
937   temozolomide therapy and mediate temozolomide resistance. *Clin Cancer Res.*
938   2009; 15(14):4622-4629.
939   **74.**   Wang J, Cazzato E, Ladewig E, et al. Clonal evolution of glioblastoma under
940   therapy. *Nat Genet.* 2016; 48(7):768-776.
941   **75.**   Erson-Omay EZ, Henegariu O, Omay SB, et al. Longitudinal analysis of treatment-
942   induced genomic alterations in gliomas. *Genome medicine.* 2017; 9(1):12.
943   **76.**   van den Bent MJ, Gao Y, Kerkhof M, et al. Changes in the EGFR amplification and
944   EGFRvIII expression between paired primary and recurrent glioblastomas.
945   *Neuro Oncol.* 2015; 17(7):935-941.
946   **77.**   Nutt CL, Mani DR, Betensky RA, et al. Gene expression-based classification of
947   malignant gliomas correlates better with survival than histological classification.
948   *Cancer Res.* 2003; 63(7):1602-1607.
949   **78.**   Gill BJ, Pisapia DJ, Malone HR, et al. MRI-localized biopsies reveal subtype-
950   specific differences in molecular and cellular composition at the margins of
951   glioblastoma. *Proceedings of the National Academy of Sciences of the United States*
952   *of America.* 2014; 111(34):12550-12555.
953   **79.**   Bhat KP, Balasubramaniyan V, Vaillant B, et al. Mesenchymal differentiation
954   mediated by NF-kappaB promotes radiation resistance in glioblastoma. *Cancer*
955   *cell.* 2013; 24(3):331-346.
956   **80.**   Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by
957   preferential activation of the DNA damage response. *Nature.* 2006;
958   444(7120):756-760.
959   **81.**   Ozawa T, Riester M, Cheng YK, et al. Most human non-GCIMP glioblastoma
960   subtypes evolve from a common proneural-like precursor glioma. *Cancer cell.*
961   2014; 26(2):288-300.
962   **82.**   Flavahan WA, Drier Y, Liau BB, et al. Insulator dysfunction and oncogene
963   activation in IDH mutant gliomas. *Nature.* 2016; 529(7584):110-114.
964   **83.**   Lu C, Ward PS, Kapoor GS, et al. IDH mutation impairs histone demethylation and
965   results in a block to cell differentiation. *Nature.* 2012; 483(7390):474.
966   **84.**   Kohanbash G, Carrera DA, Shrivastav S, et al. Isocitrate dehydrogenase mutations
967   suppress STAT1 and CD8+ T cell accumulation in gliomas. *The Journal of clinical*
968   *investigation.* 2017; 127(4):1425-1437.
969   **85.**   de Souza CF, Sabedot TS, Malta TM, et al. Distinct epigenetic shift in a subset of
970   Glioma CpG island methylator phenotype (G-CIMP) during tumor recurrence.
971   *bioRxiv.* 2017.
972   **86.**   Klughammer J, Kiesel B, Roetzer T, et al. The DNA methylation landscape of
973   glioblastoma disease progression shows extensive heterogeneity in time and
974   space. *bioRxiv.* 2017.
975   **87.**   Ellingson BM, Bendszus M, Boxerman J, et al. Consensus recommendations for a
976   standardized Brain Tumor Imaging Protocol in clinical trials. *Neuro-Oncology.*
977   2015; 17(9):1188-1198.

88. Law M, Yang S, Wang H, et al. Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging. *American Journal of Neuroradiology.* 2003; 24(10):1989-1998.

89. Albert NL, Weller M, Suchorska B, et al. Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. *Neuro Oncol.* 2016; 18(9):1199-1208.

90. Jamshidi N, Diehn M, Bredel M, Kuo MD. Illuminating radiogenomic characteristics of glioblastoma multiforme through integration of MR imaging, messenger RNA expression, and DNA copy number variation. *Radiology.* 2013; 270(1):1-2.

91. Gevaert O, Mitchell LA, Achrol AS, et al. Glioblastoma multiforme: exploratory radiogenomic analysis by using quantitative image features. *Radiology.* 2014; 273(1):168-174.

92. Zinn PO, Colen RR. Imaging genomic mapping in glioblastoma. *Neurosurgery.* 2013; 60(CN_suppl_1):126-130.

93. Weller M, Stupp R, Hegi ME, et al. Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing for malignant glioma patients in clinical practice. *Neuro-oncology.* 2012; 14(suppl_4):iv100-iv108.

94. Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ. Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? *The lancet oncology.* 2011; 12(1):83-91.

95. Van Meter T, Dumur C, Hafez N, Garrett C, Fillmore H, Broaddus WC. Microarray analysis of MRI-defined tissue samples in glioblastoma reveals differences in regional expression of therapeutic targets. *Diagnostic Molecular Pathology.* 2006; 15(4):195-205.

96. Getz G, Hofling H, Mesirov JP, et al. Comment on "The consensus coding sequences of human breast and colorectal cancers". *Science.* 2007; 317(5844):1500.

97. Weller M, Butowski N, Tran D, et al. ACT IV: AN INTERNATIONAL, DOUBLE-BLIND, PHASE 3 TRIAL OF RINDOPEPIMUT IN NEWLY DIAGNOSED, EGFRVIII-EXPRESSING GLIOBLASTOMA. Paper presented at: NEURO-ONCOLOGY2017.

98. Reardon D, Omuro A, Brandes A, et al. OS10. 3 Randomized Phase 3 Study Evaluating the Efficacy and Safety of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: CheckMate 143. *Neuro-Oncology.* 2017; 19(suppl_3):iii21-iii21.

99. International Cancer Genome C, Hudson TJ, Anderson W, et al. International network of cancer genome projects. *Nature.* 2010; 464(7291):993-998.

100. Esteve-Codina A, Arpi O, Martinez-Garcia M, et al. A Comparison of RNA-Seq Results from Paired Formalin-Fixed Paraffin-Embedded and Fresh-Frozen Glioblastoma Tissue Samples. *PLoS One.* 2017; 12(1):e0170632.

101. Erdem-Eraslan L, van den Bent MJ, Hoogstrate Y, et al. Identification of Patients with Recurrent Glioblastoma Who May Benefit from Combined Bevacizumab and CCNU Therapy: A Report from the BELOB Trial. *Cancer Res.* 2016; 76(3):525-534.

102. Kurc T, Qi X, Wang D, et al. Scalable analysis of Big pathology image data cohorts using efficient methods and high-performance computing strategies. *BMC Bioinformatics.* 2015; 16:399.

1026 **103.** Nalisnik M, Amgad M, Lee S, et al. Interactive Phenotyping Of Large-Scale
1027   Histology Imaging Data With HistomicsML. *bioRxiv.* 2017.

1028