Meta-analysis of commonly mutated genes in leptomeningeal carcinomatosis

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Background. Leptomeningeal carcinomatosis (LMC) is a rare type of cancer that settles at the meninges through metastasis of non-small cell lung cancer (NSCLC), breast cancer and melanoma. Molecular mechanism underlying LMC is not known, therefore molecular studies investigating the development of LMC are needed. Here, we aimed to identify commonly mutated genes in LMC caused by NSCLC, breast cancer, and melanoma using an in-slico approach and their interactions using integrated bioinformatic approaches/tools in this meta-analysis.

Methods. We conducted a meta-analysis using information from 16 studies that included different sequencing techniques of patients with LMC caused by three different primary cancers: breast cancer, NSCLC, and melanoma. All studies that assessed mutation information from patients with LMC were searched in PubMed, from their inception to February, 16 2022. Studies that performed NGS on LMC patients with NSCLC, breast cancer, or melanoma were included, while studies that did not apply NGS to CSF samples, did not provide information on altered genes, were reviews, editorials, or conference abstracts, or whose main goal was the detection of malignancies were all excluded. We identified commonly mutated genes in all three types of cancer. Next, we constructed a protein-protein interaction network, then performed pathway enrichment analysis. We searched National Institutes of Health (NIH) and Drug-Gene Interaction Database (DGIdb) to find candidate drugs.

Results. We found that TP53, PTEN, PIK3CA, IL7R, and KMT2D genes were commonly mutated genes in all three types of cancer via our meta-analysis that consisted out of 16 studies. Our pathway enrichment analysis showed that all five genes were primarily associated with regulation of cell communication and signaling, and cell proliferation. Other enriched pathways included regulation of apoptotic processes of leukocytes and fibroblasts, macroautophagy and growth. According to our drug search we found candidate drugs; Everolimus, Bevacizumab and Temozolomide, which interact with these five genes.

Conclusion. In conclusion, a total of 96 mutated genes in LMC were investigated via meta-analysis. Our findings suggested vital roles of TP53, PTEN, PIK3CA, KMT2D, and IL7R, which can provide insight into the molecular basis of LMC development and paving the door to the development of new targeted medicine and will encourage molecular biologists to seek biological evidence.

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Abstract

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65 analysis. Our findings suggested vital roles of TP53, PTEN, PIK3CA, KMT2D, and IL7R, which
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67 development of new targeted medicine and will encourage molecular biologists to seek biological
68 evidence.

69

Introduction

71 Leptomeningeal carcinomatosis (LMC) is a rare type of cancer that settles through metastasis from
72 a tumor in the body to the meninges. The leptomeninges are the brain and spinal cord areas that
73 contain cerebrospinal fluid (CSF). Metastatic cells involved in CSF take over the central nervous
74 system in a short time and affect the brain, spinal cord, and nerves, causing sudden neurological
75 disorders and death. Cancerous cells reach the brain through a vein and migrate from the choroid
76 plexus to the CSF. Entering the leptomeningeal region, the metastatic cancer cell can circulate
77 freely, thereby causing extensive damage to the nervous system and brain (Boire *et al.* 2017).

78

79 The mortality rate in LMC, which follows an aggressive path, is also quite high. Most solid tumors
80 are known to cause LMC, but the most common solid tumors involving leptomeninges include
81 breast, lung, and melanoma. The incidence of cancer varies between different types of cancer. It
82 is 5% to 8% among breast cancer patients (Wang *et al.* 2018) and is the most common type of fatal
83 cancer (Li Y *et al.* 2018); it ranges from 9% to 25% in lung cancer and 6% to 18% in melanomas.
84 Radiation therapy and systemic intrathecal or intravenous chemotherapy methods are used for the
85 treatment of LMC (Chahal *et al.* 2015). But the average life expectancy of tumors on the
86 membranes of the meninges with the prescribed treatments is an average of 6 months, despite the
87 longer survival time of some patients (Wang *et al.* 2018), (Özdoğan *et al.* 2004). Within the
88 framework of the unknown molecular mechanism of the disease, next-generation sequencing
89 (NGS) is being performed to find out the mutation status of circulating tumor cells (CTCs) found
90 in CSF and RNA sequencing (RNA-seq) is being performed to investigate the transcriptome
91 properties (Ruan *et al.* 2020). Currently, the diversification of cancer treatment and the
92 prolongation of patient survival have also begun to increase LMC incidence (Lee *et al.* 2013).
93 Therefore, molecular studies investigating the development of LMC are needed. The aim of our
94 meta-analysis is to explore commonly mutated genes and their interactions in LMC caused by
95 NSCLC, breast cancer, and melanoma with integrated bioinformatic approaches.

96

97 In this meta-analysis, 16 scientific papers that performed different sequencing techniques to LMC
98 patients caused by breast cancer, NSCLC, or melanoma were included. Mutated gene lists were
99 created with the given information in the detailed analysis on their genetic information obtained
100 from results and supplementary information which were available online. We categorized and
101 compared mutated genes of 3 types of primary cancers that caused LMC and found that *TP53,*
102 *PIK3CA, PTEN, KMT2D* and *IL7R* were commonly mutated in all three types of cancers. To reveal
103 protein interactions, we constructed a protein-protein interaction network and performed pathway
104 enrichment analysis. Our results suggested several key pathways such as regulation of cell
105 communication and signaling, and cell proliferation might have a role in LMC development and
106 three candidate drug options, Everolimus, Bevacizumab and Temozolomide, that can be used for
107 LMC treatment.

108

Materials & Methods

110 Guideline for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)
111 statement was followed in the conduct and reporting of this meta-analysis (Page *et al.* 2021).

112

Literature search

114 We carried out a literature search on PubMed database using Medical Subject Headings (MeSH)
115 indexing terms and keywords associated with leptomeningeal carcinoma (Supplementary File 1).
116 Without any additional publication restrictions, the search was carried out in English. This analysis
117 covered papers published between February 16, 2012, and February 16, 2022 that provided
118 information on mutated genes among patients with LMC. This time frame was chosen to include

119 more modern studies, reflecting the significance of sequencing technologies. As part of the review
120 process, references from publications that were included were also scanned; however, a search of
121 the grey literature was not performed.
122
123 **Inclusion and Extraction Criteria**
124
125 Clinical studies that performed NGS to LMC patients caused by NSCLC, breast cancer, or
126 melanoma were included.
127
128 Studies were excluded if they did not apply NGS to CSF samples, lacked to provide accurate
129 information regarding mutated genes, were reviews, editorials, or conference abstracts, or if their
130 primary intention was the detection of tumors. Studies with objectives that did not entail using
131 human patients were also excluded after evaluation (Figure 1). An analysis path has been followed
132 as described in the following steps and shown in Figure 2.
133
134
135
136
137
138 **Data selection**
139
140 Since the data and content used in our study are derived from the tables and figures that have
141 already been interpreted in the articles, no loss, modification or editing has been carried out in our
142 results.
143
144 The type of article, the title, and the abstract were checked in order to find studies from the
145 literature search that might be relevant. Two reviewers (IC and EK) independently evaluated the
146 entire texts of the articles for pertinent data and then selected which papers to include.   The
147 following information was extracted: first author, primary tumor site, sequencing technique,
148 mutated genes and their patient relevance. Whenever there was a difference of opinion during the
149 screening and extraction processes, a solution was found through discussion and with the advice
150 of a third reviewer (ZTK).
151
152 **Identification and categorization of mutated genes**
153 16 articles were categorized based on their primary tumor location: 11 NSCLC-LMC, 3 breast-
154 LMC and 2 melanoma-LMC (Table 1). The causes of the formation of the three types of primary
155 cancer and the prevalence in groups contain differences within themselves. Therefore, the detailed
156 characteristic information of the patients (age, gender, primary tumor mutation, treatment before
157 LMC diagnosis and NGS) have been neglected in the selected studies, whereas the mutated genes
158 detected by NGS applied to CSF samples of LMC patients were included. This information was

159   obtained from "results" or "supplementary information" of each article. Mutated genes observed
160   in only one patient were discarded. Further analysis was performed with the mutated genes (n≥2,
161   n=patient number) shown in Figure 3.

162

163   **Construction of protein-protein interaction (PPI) network and pathway enrichment analysis**
164   PPI network construction
165   For each category of mutated genes (Figure 3), PPI network was constructed using STRING
166   database (http://string-db.org/) (Szklarczyk *et al.* 2019) version: 11.5. Within basic settings, Full
167   STRING network and confidence boxes were checked.

168

169   Pathway enrichment analysis
170   g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) (Raudvere *et al.* 2019) version
171   e105_eg52_p16_e84549f (database updated on 03/01/2022) was used for pathway enrichment
172   analysis. Reimand *et al* 2019 protocol was followed. Within the options section following boxes
173   were checked: Homo sapiens and ordered query. According to Reimand et al. within the options
174   section, after selecting Homo sapiens as a specific organism, ordered query box was checked.
175   Then, for GO analysis, section biological process (GO: BP) and for biological pathways section
176   Reactome database was chosen. For advanced options, "only annotated genes" as statistical
177   domain scope, "g:SCS threshold" as significance threshold and "0.05" as user threshold were
178   selected.

179

180   **Visualization and interpretation of pathway enrichment analysis results**
181   String output was uploaded to Cytoscape (https://cytoscape.org) (Shannon *et al.* 2003) version
182   3.9.1 in order to edit. Then, the result files of g:Profiler were uploaded to Cytoscape and a pathway
183   network was constructed to visualize and interpret the output. Within the software, plug-in
184   EnrichmentMap was used. According to Reimand et al. in the input panel of EnrichmentMap
185   number of nodes section, FDR Q value cutoff was set to 0.01. For the number of edges, predefined
186   parameters were used.

187

188   **Investigation of mutations**
189   Mutation information of commonly mutated five genes (TP53, PIK3CA, PTEN, IL7R, and
190   KMT2D) were retrieved from 16 articles that were included in this meta-analysis. Databases
191   dbSNP (https://www.ncbi.nlm.nih.gov/snp/), Catalogue of Somatic Mutations in Cancer
192   (COSMIC) (https://cancer.sanger.ac.uk/cosmic), National Cancer Institute (NCI) Genomic Data
193   Commons (GDC) data portal (https://portal.gdc.cancer.gov/), and ClinVar
194   (https://www.ncbi.nlm.nih.gov/clinvar) were used to search the mutations.

195

196   **Drug search**
197   National Institutes of Health (NIH) (https://www.nih.gov/) was used to find FDA (Food and Drug
198   Administration) approved drugs that treat NSCLC, breast cancer, melanoma, and brain tumor.

199   Drug-Gene Interaction Database (DGIdb) (https://www.dgidb.org/) (Freshour *et al.* 2021) version
200   v4.2.0 - sha1 was used to find potential drugs that interact with selected genes. Clinicaltrials.gov
201   (https://clinicaltrials.gov/) database was used to search for present clinical status of candidate
202   drugs.
203
Results
205   After thorough research, 221 studies were selected. There were no duplicate results as PubMed
206   was used for the search. Among the 221 articles that were examined for their titles and abstracts,
207   only 23 were qualified for a full-text review. Finally, 16 studies were included and assessed in this
208   meta-analysis as shown in Figure 1.
209

Identification of mutated genes in LMC patients according to their primary tumor sites
211   In order to observe mutated genes according to primary tumor sites we created a venn diagram.
212   Specific patient characteristic information such as age, gender, primary tumor mutation, treatment
213   prior to LMC diagnosis, and treatment before sequencing were neglected because of the limited
214   number of studies. Moreover, we wanted to focus on revealing the commonly mutated genes
215   causing LMC out of the three primary cancers, NSCLC, breast cancer and melanoma. Hence, the
216   current study includes mutated genes identified by NGS applied to CSF samples of LMC patients.
217   A total of 96 genes were found to be mutated in all cancer groups, which distributed as 87 genes
218   in NSCLC, 24 in breast and 13 in melanoma. 16 of these genes were shared between NSCLC and
219   breast; 11 of them were common between NSCLC and melanoma; and 6 of them were shared
220   between breast and melanoma. We found out that five genes (*TP53*, *PIK3CA*, *PTEN*, *IL7R* and
221   *KMT2D*) were commonly mutated in each of the cancers (Fig. 3).
222

PPI network construction of mutated genes in each primary cancer type
224   We continued our analysis with genes that are mutated in more than two patients for each primary
225   tumor site, which are illustrated in Figure 4a-c. In order to investigate the potential interactions of
226   genes mutated in NSCLC-LMC patients, we constructed a PPI network using STRING database.
227   This PPI network contained 87 nodes and 1181 edges (Fig 4d). Whereas 11 nodes and 16 edges
228   were found in the PPI network of breast-LMC (Fig 4e), and only two nodes and one edge were
229   found in melanoma-LMC PPI network (Fig 4f).
230

PPI network and pathway enrichment analysis of commonly mutated genes in all three
cancer groups
233   Next, we maintained our analysis with mutated genes that were observed in at least two primary
234   tumor sites (Figure 5a). We wondered if we could reveal a mechanism that causes metastasis from
235   all three types of cancer to the meninges of the brain by investigating mutated genes. Therefore,
236   we carried on our analysis with five genes that are commonly mutated in all three primary tumor
237   sites. To see protein-protein interactions of TP53, PIK3CA, PTEN, IL7R and KMT2D, STRING
238   database was used to construct a PPI network. PPI network included 5 nodes and 6 edges (Fig

239   5b). Surprisingly, IL7R did not make an interaction with any other proteins. Next, we performed
240   pathway enrichment analysis in order to investigate the pathways that these genes were involved
241   in (Fig 5c). The enriched GO pathways were presented in Supplementary Figure 1. There were 7
242   pathways with some having subgroups.

244   All five gene products were primarily associated with positive regulation of cell communication,
245   positive regulation of signaling, positive regulation of response to stimulus, and positive regulation
246   of signal transduction pathways. In addition, they are present in cell population proliferation and
247   regulation of cell population proliferation pathways. In developmental growth and growth
248   pathways TP53, PTEN, PIK3CA, and KMT2D were involved. TP53, PTEN, and IL7R had roles
249   together in regulation of lymphocyte apoptotic process, lymphocyte apoptotic process, regulation
250   of leukocyte apoptotic process, and leukocyte apoptotic process pathways. Whereas "PIP3
251   activates AKT signaling" pathway involved TP53, PTEN, and PIK3CA. Moreover, TP53 and
252   PIK3CA were present in regulation of fibroblast apoptotic process and fibroblast apoptotic process
253   as well as negative regulation of macroautophagy.

255   **Investigation of mutations in commonly mutated genes**

257   We extracted the mutation information of five commonly mutated genes in all primary tumor sites
258   from articles included in this meta-analysis. Among 16 studies that were selected only 11 of them
259   presented the mutation information of the genes. However, in these 11 studies, not all mutation
260   information was reported. You can access the given mutation information of commonly mutated
261   five genes from the supplementary file 4. We found 15 mutations in TP53, three mutations in
262   PIK3CA and PTEN, two mutations in IL7R, and 6 mutations in KMT2D. We searched each
263   mutation in dbSNP, COSMIC database, ClinVar, and GDC data portal. Some of these mutations
264   were registered in the above-mentioned databases, whereas some were reported by only the articles
265   that were analyzed in ours.

267   TP53 mutations R248Q and R248W are known as hotspot mutations, located in the DNA-binding
268   domain of the TP53 gene. The R248Q mutation results in a diminished binding of DNA and a
269   decrease in the activation of its targets, which hinders the transactivation of wild-type TP53 and
270   leads to resistance against apoptosis and G1 arrest in cell culture, as evidenced by several research
271   articles (Hanel et al. 2013), (Dearth et al. 2007), (Boettcher et al. 2019). R248Q was seen in 4
272   NSCLC-LMC and melanoma-LMC patients, while according to the GDC data portal there were 8
273   breast and 6 lung cancer NCI cases that were registered. On the other hand, the R248W mutation
274   was shown to reduce the transactivation of TP53 targets, promote cell proliferation in culture, and
275   lead to an increase in tumorigenesis in mice, which was accompanied by decreased activation of
276   ATM and an increase in genetic instability (Song et al. 2007), (Willis et al. 2004). Although the
277   R248W mutation was observed in only one NSCLC-LMC patient, it was found in 9 breast, 5 lung,
278   and 3 skin cancer NCI cases registered in GDC data portal. Another hotspot mutation among LMC

279 patients was PIK3CA E545K, which is located in the PIK helical domain and results in increased
280 AKT and MEK1/2 phosphorylation, cell survival independent of growth factors, and cell
281 transformation in culture (Dogruluk et al. 2015), (Ng et al. 2018). 6 NSCLC-LMC and melanoma-
282 LMC patients had this mutation, whereas 83 breast, 23 lung, and 2 skin cancer NCI cases were
283 registered. Two KMT2D mutations, Q3278del and Q3861del, were seen in 6 NSCLC-LMC
284 patients, however none of these mutations were registered in any mutation database.

285

286 **Drug search**
287 We investigated the drugs that could be used in common to target mutated genes in all three types
288 of cancer. Therefore, FDA approved drugs that treat NSCLC, breast cancer, melanoma, and brain
289 tumors were found with NIH (Figure 6). Among these, drugs that have capability to penetrate the
290 blood-brain barrier (BBB) to reach tumor site were selected and searched in DGIdb to look for
291 interaction with the selected genes (*TP53, PIK3CA, PTEN, IL7R* and *KMT2D*). We then used
292 Clinicaltrials.gov (https://clinicaltrials.gov/) database to search for present clinical status of
293 candidate drugs. While no drugs were found to interact with IL7R and KMT2D, 3 drug candidates,
294 Everolimus, Bevacizumab and Temozolomide, had interactions with TP53, PIK3CA, and PTEN,
295 which are listed in Table 2.

296

Discussion

298 LMC is a life-threatening consequence of solid tumors that has a poor prognosis regardless of
299 treatment (Pellerino *et al.* 2020). Because effective treatment strategies against primary tumors are
300 developing every day, LMC incidences are increasing (Lee *et al.* 2013). Although the number of
301 sequencing applications to LMC patients has been increasing in recent years, they are mainly
302 aimed at facilitating the diagnosis of the disease. There are limited studies that investigated
303 differentially expressed genes (DEGs) in LMC patients in vitro. One study revealed *CEACAM6*
304 gene, which had elevated expression and correlation with cellular migration in NSCLC cell line
305 (Li Y *et al.* 2021). Therefore, it is vital to establish biomarkers and the exact molecular mechanism
306 for the prognosis and treatment of LMC. In this study, mutated genes of 3 types of primary cancer
307 that caused LMC were analyzed with bioinformatics tools to determine related pathways which
308 may be involved in the development of LMC.

309

310 According to this meta-analysis, *TP53, PIK3CA*, *PTEN*, *KMT2D*, and *IL7R* genes were commonly
311 mutated in LMC patients caused by NSCLC, breast, and melanoma. The PPI network constructed
312 with STRING showed that there is an interaction within these gene products except IL7R. Even
313 though IL7R lacked direct interaction and stayed alone, it was observed that IL7R was involved
314 in the enriched pathways with other four genes. According to the pathway enrichment analysis
315 performed with g:Profiler, apoptotic processes of lymphocytes and leukocytes, regulation of
316 signaling pathways, growth, fibroblast apoptotic process, proliferation of cell population, AKT
317 signaling and regulation of macroautophagy were found to be relevant with occurrence of LMC.
318 These findings also pointed to important clues about molecular interactions in LMC development.

319

320   All five genes were involved in both regulation of signaling and cell population proliferation
321   pathways. Signaling pathways are responsible for many vital characteristics of cells like cell-to-
322   cell communication, cell proliferation, cell growth, cell death, and cellular movement. In normal
323   cells, cell proliferation and cell-to cell communication are tightly regulated by PI3K-AKT and
324   RAS-ERK pathways. Due to any alterations in one of these five genes, signaling pathways may
325   act on cancer progression, in which cancer cells will excessively proliferate (Sever *et al.* 2015).
326   One of these genes, PIK3CA encodes the p110α subunit of Class IA PI3K, and the other PTEN
327   negatively regulates PI3K pathway by acting as an antagonist (Yang *et al.* 2019). Aberrant PI3K
328   signaling, loss of function mutations of *PTEN*, and *PI3K* mutations have the ability to promote
329   invasion, metastasis and immune cell modulation in brain metastasis (Crespo *et al.* 2016). *PIK3CA*
330   mutations are among the most frequently seen genetic changes in breast cancer, occurring in about
331   25–45% of cases. Studies have revealed that *PIK3CA* mutations are more frequent in estrogen
332   receptor positive breast cancer (Samuels et al. 2010). E545K is one of the most common alterations
333   of *PIK3CA* seen in breast cancer patients (Fusco et al. 2021). *PTEN* is another most frequently
334   changed gene in breast cancer. In 5-10% of breast cancer patients, frameshift mutations cause loss
335   of function of PTEN (Carbognin et al. 2019). In NSCLC, the PI3K/Akt/mTOR pathway has been
336   heavily implicated in both tumorigenesis and the progression of disease. Somatic mutations of
337   *PIK3CA* and amplifications of *PIK3CA* are frequently found in patients with NSCLC. According
338   to a large cohort study, certain NSCLC subtypes, such squamous cell carcinoma, exhibit a higher
339   prevalence of *PIK3CA* mutations than others. Among those, the most prevalent mutation was
340   E545K exon 9 (57.1%) (Tan et al. 2020). *PTEN* genetic changes are seen in lung cancer at a low
341   frequency (2–7%). The prevalence of PTEN protein loss is significantly higher compared to the
342   modest frequency of genetic changes. It has been reported in more than 40% of NSCLC cases
343   (Gkountakos et al. 2019). In another cohort study, sequencing findings suggested that PI3K
344   pathway abnormalities may be linked to an increased risk of developing NSCLC-LMC (Fan et al.
345   2018). Research has demonstrated that patients with melanoma may develop acquired resistance
346   to anti-cancer therapy due to the activation of the PI3K signaling pathway, either as a result of
347   *PTEN* loss or *PIK3CA* mutations (Tran et al. 2021). *PTEN* has been demonstrated to be inactivated
348   or deleted in up to one-third of melanomas (Dong et al. 2014).

349

350   In addition to these, tumor suppressor TP53, halts cell proliferation in response to various stress
351   conditions such as DNA damage and is also involved in DNA repair, cell cycle, cell metabolism,
352   apoptosis, senescence, and autophagy (Liang *et al.* 2010). *TP53* gene mutations are prevalent in
353   breast cancer, occurring in 20-30% of cases, and have been associated with more aggressive
354   subtypes of the disease, as evidenced by studies (Shahbandi et al. 2020). Truncating mutations in
355   TP53 can potentially promote tumor growth. For example, the TP53 exon 6 truncating mutant
356   R196* has been shown to facilitate tumor cell growth and metastasis. (Chen et al. 2022). *TP53*
357   gene mutations are found in approximately 50% of cases of NSCLC. (Mogi et al. 2011). Studies
358   have suggested that *TP53* mutations are associated with a poor prognosis in NSCLC, as they can

359 disrupt the normal function of the TP53 protein (Chen et al. 2022). It was reported that TP53
360 mutations are found in approximately 15-25% of melanoma cases, which are linked to a higher
361 risk of recurrence and shorter overall survival (DeLeon et al. 2020). These mutations may also
362 play a crucial role in the development of metastatic melanoma (Loureiro et al. 2020). In the concept
363 of cell survival and death, interaction of PI3K/AKT pathway and TP53 have substantial roles.
364 Several studies indicated that TP53 has the ability to activate PTEN by influencing PI3K (Abraham
365 *et al.* 2014). A variety of different mechanisms also stimulate cell proliferation. PI3K/AKT and
366 JAK/STAT pathways can be triggered by IL7R, which is the receptor of IL7, an important
367 cytokine. In addition to that, IL7 plays a fundamental role in development of lymphocytes
368 (Campos *et al.* 2019). Tumor infiltrating cells (TILs) that are found in the tumor microenvironment
369 inhibit progression of cancer and are successful in active cancer immunotherapy (Zhang *et al.*
370 2019). IL7 is a proper target for improving immune system function. It has the ability to re-
371 establish the immune system, enhance T cell function in vivo, and oppose the immune system's
372 suppressive network (Gao *et al.* 2015). Epigenetic mechanisms also regulate cell proliferation and
373 survival. KMT2D, which is a histone methyltransferase catalyzes the specific lysine residues on
374 histones and proteins. It is deleted and mutated in a variety of cancers, primarily melanoma and
375 lung cancer, then breast cancer (Dhar et al. 20121). KMT2D takes part in TP53 and PI3K pathways
376 as an epigenetic modifier that is responsible for stimulating gene expression (Lv *et al.* 2018).
377 Mutations in *KMT2D* suggest epigenetic mechanisms may be involved in LMC development
378 whereas it is not very surprising to see *TP53*, *PIK3CA*, and *PTEN* gene mutations since they have
379 roles in multiple pathways in our body and frequently mutated in multiple cancer types.
380
381 According to pathway enrichment analysis, TP53, PTEN, PIK3CA and KMT2D also take place in
382 pathways related with growth. Uncontrolled cell growth may occur due to any mutations according
383 to tumor type. With the presence of cytokines and growth factors many apoptotic pathways can
384 have an effect on the tumor microenvironment to form an anti-tumor response (Zhu *et al.* 2020),
385 (Anderson *et al.* 2020). The other two pathway classes consist of regulation of leukocyte and
386 lymphocyte apoptotic processes and fibroblast apoptotic processes. Apoptosis, which is controlled
387 cell death, is important for immune function. Strict regulation of apoptosis is vital for lymphocytes
388 and leukocytes, as the altered regulation and mutations in *TP53*, *PTEN*, *IL7R* genes can result in
389 cancer formation (Zhu *et al.* 2019), (Carrero *et al.* 2006). Progression of cancer is triggered by the
390 intense relation of stromal cells found in the tumor microenvironment. Moreover, the last enriched
391 pathway involved the negative regulation of macroautophagy. Macroautophagy, also referred as
392 autophagy, is important for the breakdown of old proteins and damaged organelles, as well as
393 keeping balance of the cellular environment. Autophagy can facilitate tumorigenesis by
394 contributing to cancer-cell proliferation, but it also acts as a tumor suppressor by inducing
395 apoptosis. Our analysis showed that TP53 and PIK3CA negatively regulate macroautophagy.
396 Although PTEN was not involved in this pathway according to our analysis, several studies
397 showed that PTEN and TP53 alterations are frequently observed in brain tumors and have roles in
398 regulation of autophagy (Kaza *et al.* 2012). However, it remains unclear whether it contributes to

399 tumor progression or suppression due to its dual effects in LMC (Shi *et al.* 2021), (Yun *et al.*
400 2018).

401

402 Further investigation of mutations in TP53, PIK3CA, PTEN, KMT2D, and IL7R were also
403 performed in this study. Three hotspot mutations were seen in LMC patients, TP53 R248Q, TP53
404 248W, and PIK3CA E545K. In a study consisting of 23 cases of CNS metastatic breast cancer,
405 high frequency of TP53 mutations were detected. Those mutations included R248Q, R248W, and
406 R196* (Lo Nigro et al. 2011). In one research article, PIK3CA gene mutations, such as E545K
407 were found in NSCLC-CNS metastatic lesions (Nicos et al. 2016). Recent studies demonstrated
408 that ER+/HER2- breast cancer patients may be more likely to develop CNS metastasis if they have
409 PIK3CA-activating mutations. Brain metastases were substantially more frequent in patients with
410 PIK3CA mutations, such as E545K among 307 patients with ER+/HER2 metastatic breast cancer
411 (Batalini et al. 2020). Although some of the mutations discussed in this meta-analysis have some
412 proof to induce brain metastasis, these mutations that were investigated in this research study was
413 very limited to their availability in the papers that formed the basis of this study. There could still
414 be many different mutations in the five genes revealed in this study and their togetherness could
415 yet be important for the development of LMC. That is why, the effect the mutational
416 differences/commonalities between these genes represented in this paper could further be
417 investigated in future studies. Moreover, it will be groundbreaking if any signature mutations were
418 discovered to identify LMC which would open the gates for developing new treatment
419 opportunities.

420

421 In recent years, molecular targeted cancer therapy is in demand. NSCLC, breast cancer and
422 melanoma, which are primary tumor types of LMC may have marker mutations such as *EGFR*,
423 *HER2* and *BRAF* respectively. There are targeted therapy options for these kinds of mutations.
424 Unfortunately, there is still no gold standard in treatment of LMC. Current management of this
425 disease includes radiotherapy, systemic and intrathecal chemotherapy that is commonly described
426 in the literature as cytarabine and methotrexate (Lee *et al.* 2016). One concept that should be kept
427 in mind when treating LMC is that the drugs must penetrate BBB to reach the tumor site.

428

429 Our analysis revealed that, among 179 FDA approved chemotherapy drug options for NSCLC,
430 breast cancer, and melanoma, only 1 of them was common but it could not cross BBB. There were
431 a limited number of BBB-penetrating drug options for NSCLC-breast cancer and NSCLC-
432 melanoma. To find out drugs that could both penetrate BBB and target these 5 genes, FDA
433 approved brain tumor drugs were examined. Among 7 drugs, Everolimus, Bevacizumab, and
434 Temozolomide that target PTEN, PIK3CA, and TP53 respectively are revealed as targeted therapy
435 candidates for LMC treatment with patients bearing mutations in any of these genes. In addition
436 to these, there are many drugs that are under clinical investigation for NSCLC, breast cancer and
437 melanoma that target altered genes. Disulfiram that targets TP53 and Buparlisib that target

438 PIK3CA and PTEN can be given as examples. Hopefully, in the near future clinical trials involving
439 differential use of such drugs can shed light to treatment of LMC.
440
441 During the study some limitations were encountered. As this meta-analysis was composed of
442 publicly available articles, it also limited the availability of the sequencing information about
443 LMC. Because LMC is not that frequently observed, cancer databases like The Cancer Genome
444 Atlas (TCGA) have no information about LMC that can be further analyzed. The other is that the
445 information about mutations of patients' primary tumor sites was inadequate. Moreover, effects of
446 commonly mutated genes were not further explored due to lack of experimental research. Despite
447 these limitations, we hope that this study will be a pioneer in future studies of LMC and that this
448 lack of information will be eliminated.
449

450 ## Conclusions
451 In conclusion, a total of 96 mutated genes in LMC were investigated via integrated bioinformatic
452 analysis to further understand the molecular mechanism of LMC. Considering the vital roles of
453 genes commonly mutated in each primary tumor type, further research into their precise pathways
454 in the tumorigenesis and prognosis of LMC, particularly TP53, PTEN, PIK3CA, KMT2D, and
455 IL7R, may be pursued. Due to unknown aspects of molecular mechanism of LMC, larger cohort
456 studies with multiple omics approaches, development of new drug candidates and combinatorial
457 drug trials are much needed.
458

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460 The meta-analysis uses already registered and published data. For this reason, the data analyzed
461 here do not require further registry.
462

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Figure 1

Flow diagram of the study selection

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

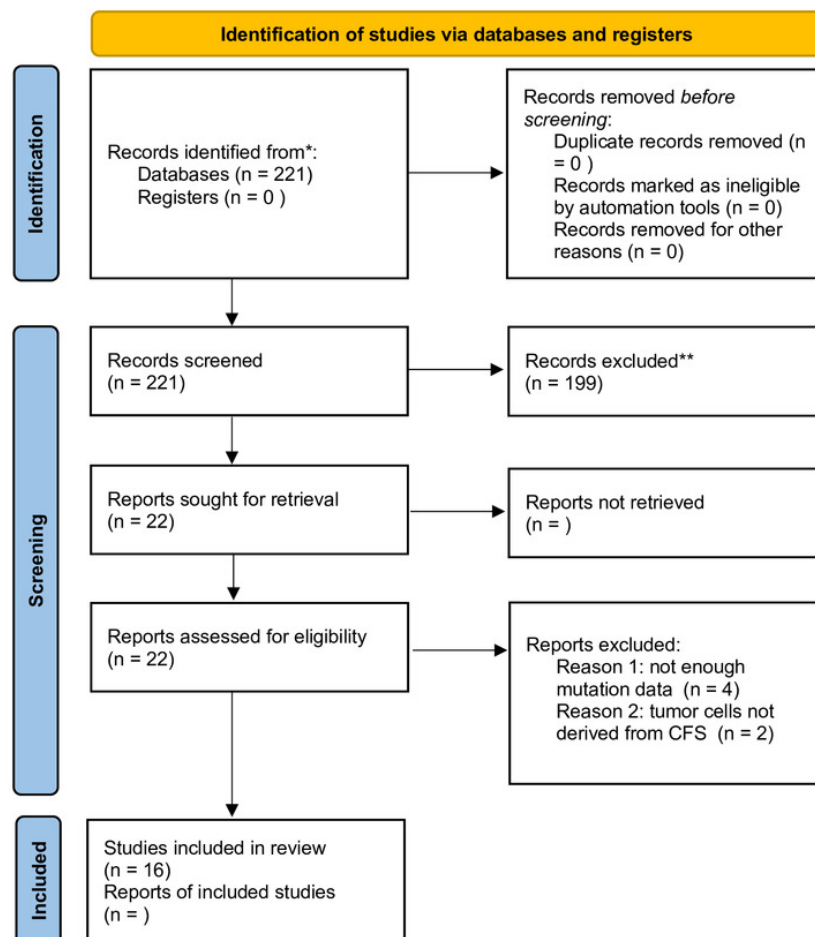



Figure 2

Systematic workflow of the methodology.

Systematic workflow of the methodology. The meta-analysis was conducted using information obtained from 16 studies involving different sequencing techniques of patients with LMC caused by three different primary cancers (breast cancer, NSCLC, and melanoma). Then, a protein-protein interaction network was created, and pathway-enrichment analysis was performed. Candidate drugs were investigated via the Drug-Gene Interaction Database and interpreted with the genes obtained as a result of the analysis. LMC, leptomeningeal carcinomatosis; NSCLC, non-small cell lung cancer

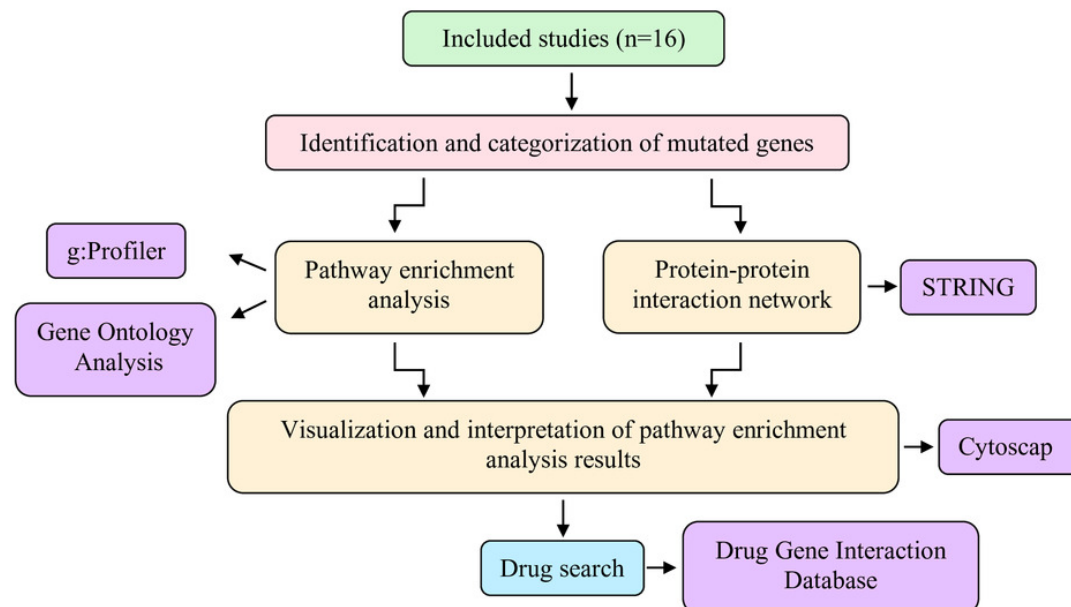



Figure 3

Venn diagram showing mutated genes in LMC patients according to their primary tumor sites.

When all the genes reported were plotted in a Venn diagram, five genes appeared to be in common. These five genes shown in the red box were TP53, PTEN, PIK3CA, KMT2D and IL7R were mutated commonly in all three primary tumor sites (NSCLC, breast cancer, melanoma). LMC, leptomeningeal carcinomatosis; NSCLC, nonsmall cell lung cancer

Melanoma-LMC

NRAS

GNAQ
RET
BRAF
APC
CDKN2A
MET

TP53
PIK3CA
KMT2D
PTEN
IL7R

PMS2

65

MAP3K1
TET2
NOTCH2
EPHB6
CLTCL1
ARID2
ALDH2

Breast-LMC

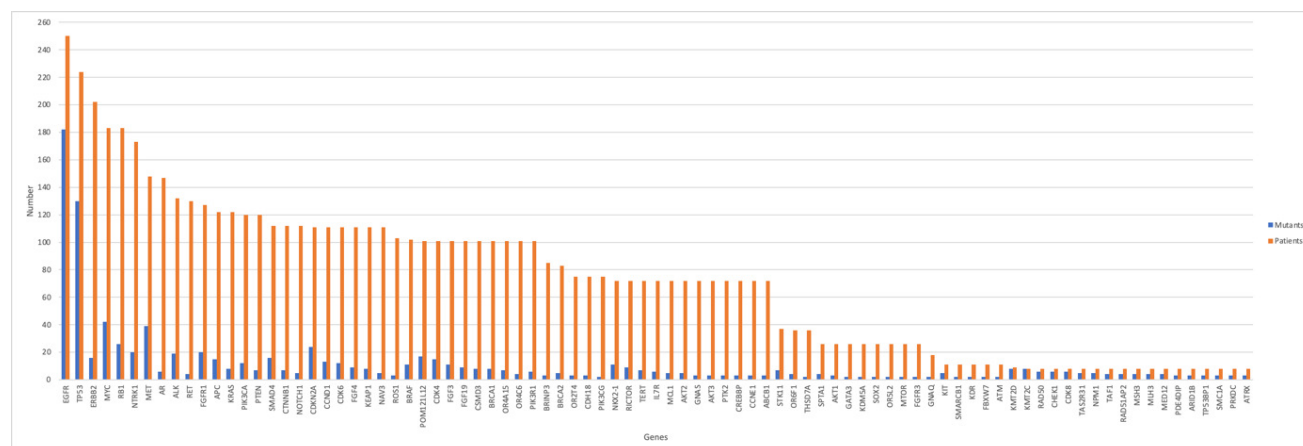
NSCLC-LMC

KDM5A
GATA3
ROS1
GNAS
AKT3
PDE4DIP
ARID1B
BRCA2
KIT
KMT2C
CDK6

EGFR, MYC, RB1, NTRK1, FGFR1,
ALK, POM121L12, ERBB2, SMAD4,
CDK4, CCND1, FGF3, NKX2-1, FGF4,
FGF19, RICTOR, KRAS, KEAP1,
CSMD3, BRCA1, CTNNB1, OR4A15,
TERT, STK11, AR, RAD50, CHEK1,
CDK8, NOTCH1, NAV3, MCL1, AKT2,
TAS2R31, NPM1, OR4C6, OR6F1,
SPTA1, TAF1, RAD51AP2, MSH3,
MLH3, MED12, BRINP3, OR2T4,
CDH18, PTK2, CREBBP, CCNE1,
ABCB1, AKT1, TP53BP1, SMC1A,
PRKDC, ATRX, PIK3CG, THSD7A,
SOX2, OR5L2, MTOR, FGFR3,
SMARCB1, KDR, FBXW7, ATM,

Mutated genes and their interactions with each other in each primary cancer type.

Bar graph showing mutated genes in A. NSCLC-LMC, B. Breast-LMC and C. Melanoma-LMC patients (n≥2). D. PPI network of mutated genes in NSCLC-LMC. Among 87 proteins 12 of them did not interact with any other proteins. E. PPI network of mutated genes in breast-LMC. Among 11 proteins only one of them (ALDH2) did not make any interaction, while CLTCL1 and EPHB6 only interacted with each other. F. PPI network of mutated genes in melanoma-LMC. NRAS and BRAF interacted with each other. PPI, proteinprotein interaction; LMC, leptomeningeal carcinomatosis; NSCLC, non-small cell lung cancer; n=patient number.

A.

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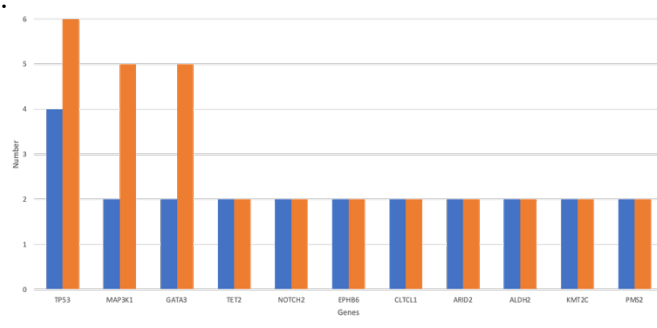
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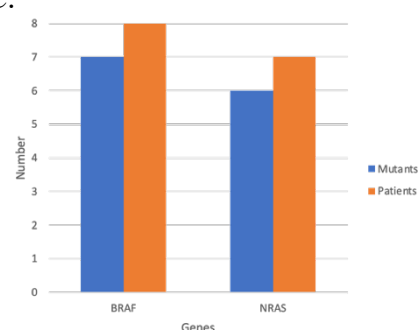
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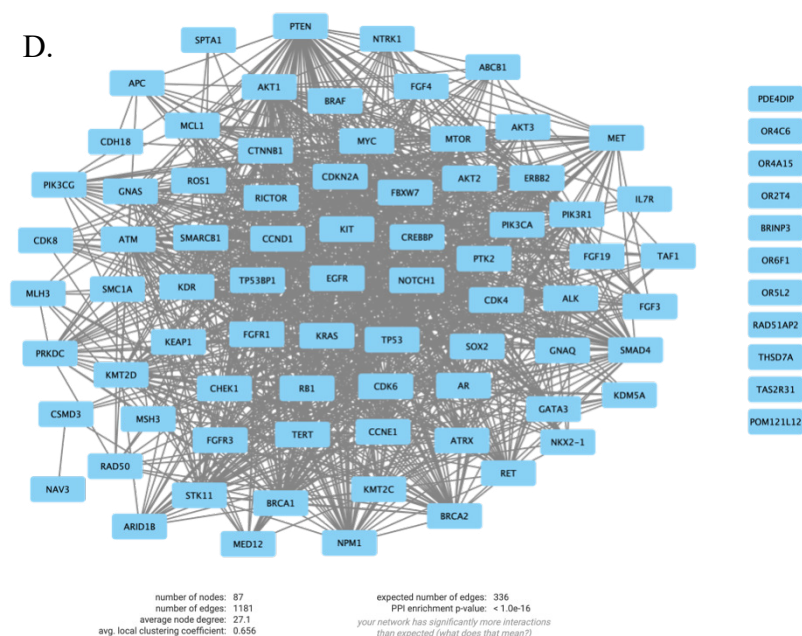
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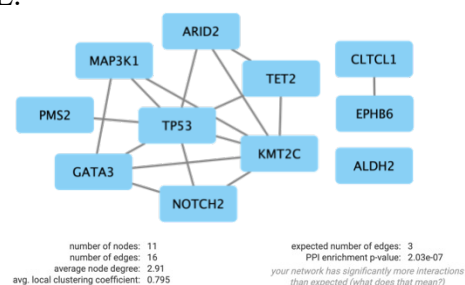
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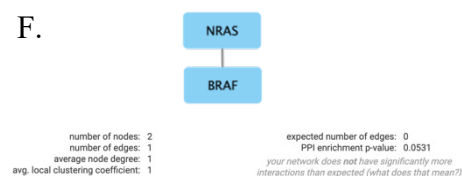

PPI network and pathway enrichment analysis of commonly mutated genes in all three cancer groups.

A. Bar-chart showing mutated genes in at least two primary tumor sites (n≥2). Five genes (TP53, PTEN, PIK3CA, IL7R, and KMT2D) were mutated in all three tumor sites. B. PPI network of mutated genes appeared in all three cancer groups. All four proteins were interacted with each other, with the exception of IL7R. C. Visualization of pathway enrichment analysis of mutated genes among all three cancer groups using Cytoscape. All five genes were involved in signaling and cell population proliferation pathways. TP53, PTEN, PIK3CA, and KMT2D had roles in pathways regarding growth. TP53, PTEN, and PIK3CA were involved in AKT signaling pathway. TP53, PTEN, and IL7R were involved in lymphocyte and leukocyte apoptotic processes. Finally, TP53 and PIK3CA had roles in fibroblast apoptotic processes and regulation of macroautophagy. n=patient number. NSCLC, non-small cell lung cancer.

A.

B.

C.

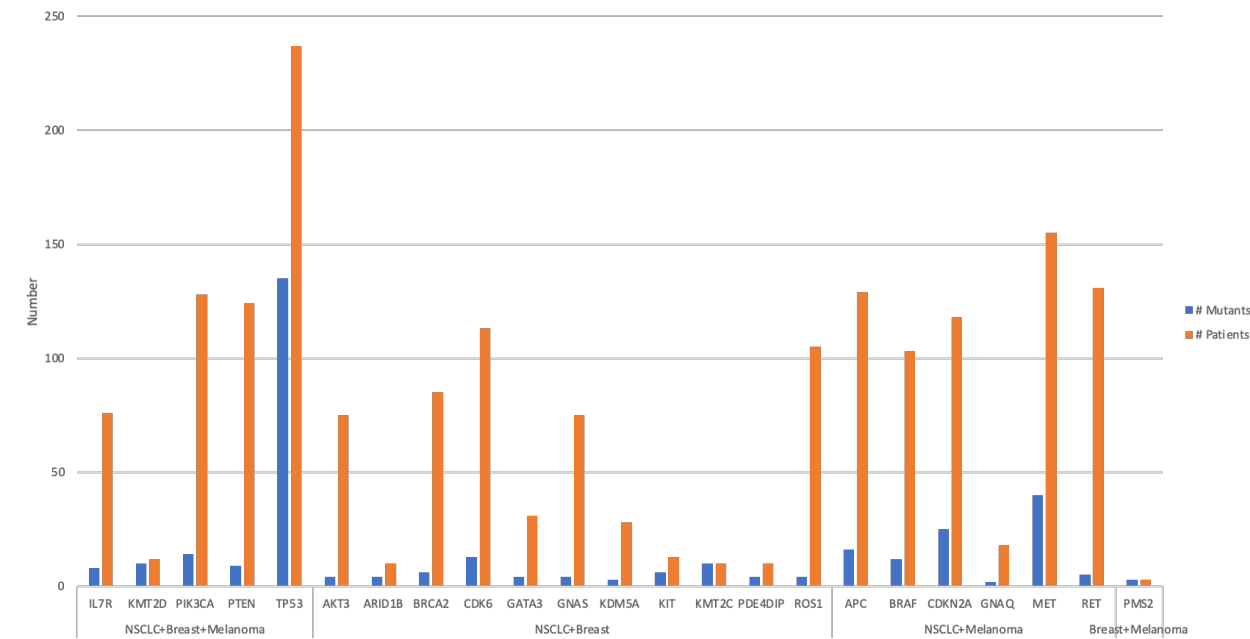
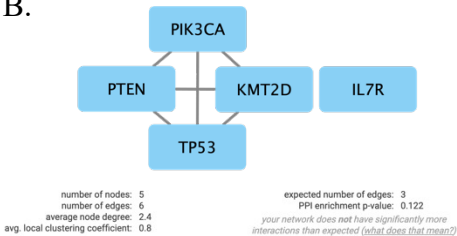
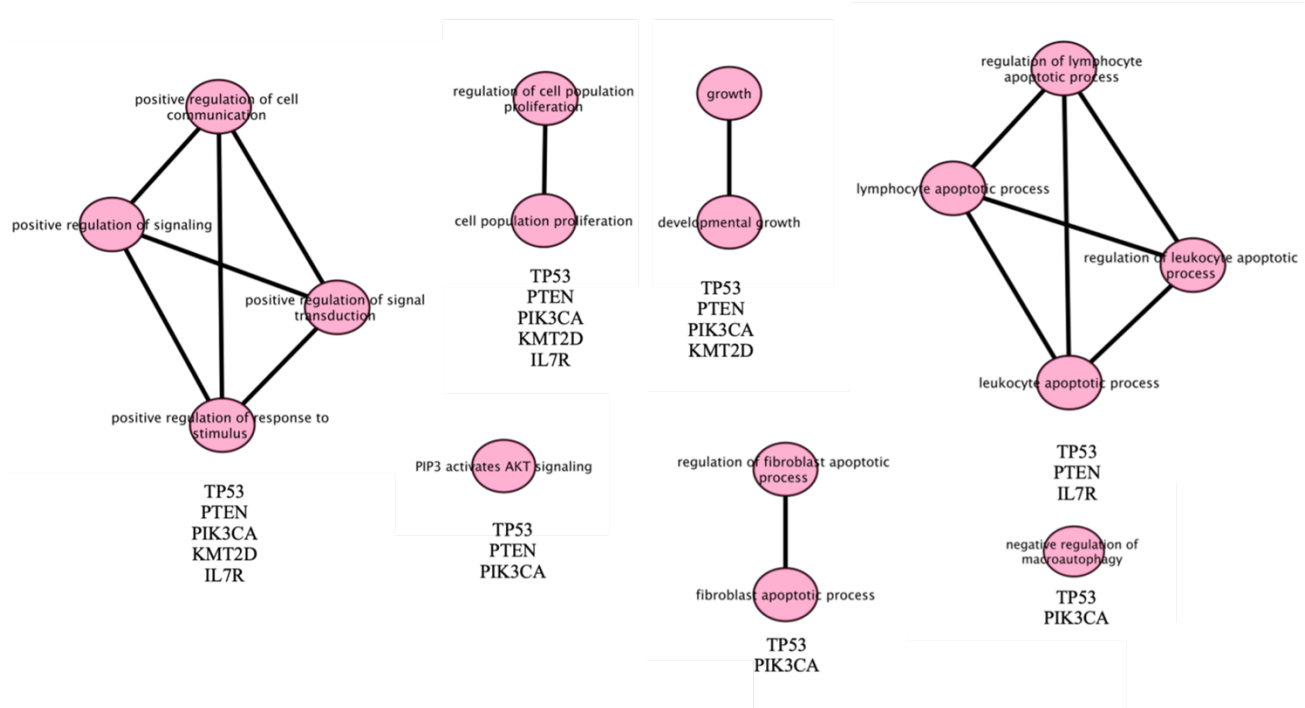
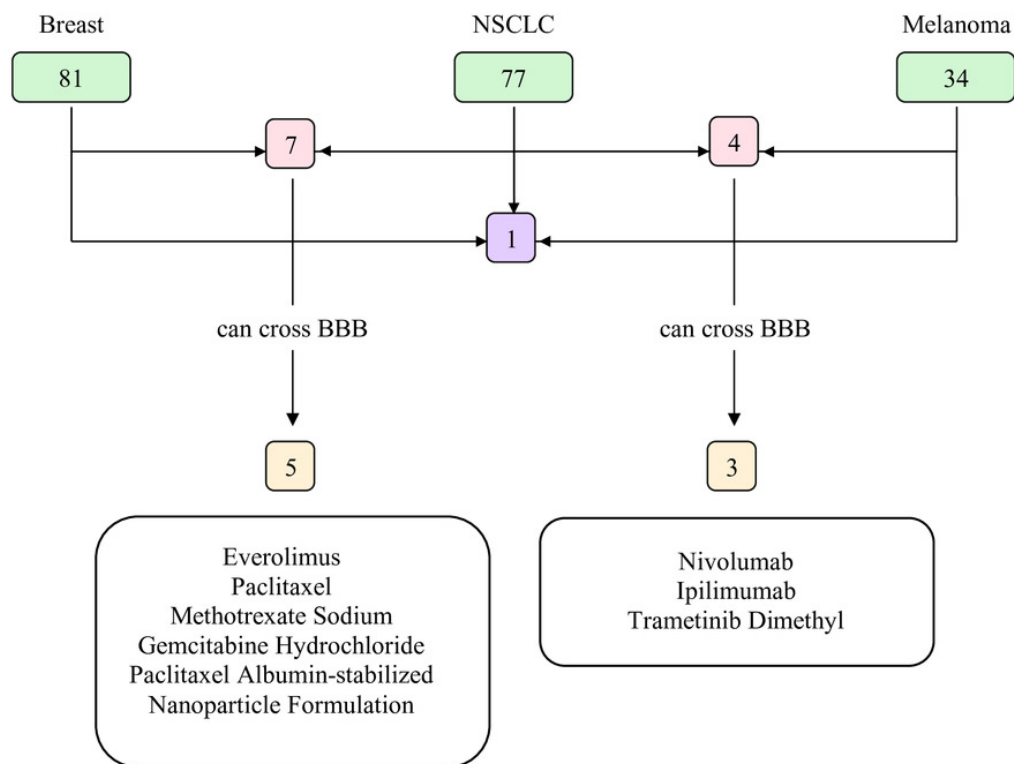



Figure 6

Food and Drug Administration (FDA) approved drugs.

Number of FDA approved drugs used for treatments of breast cancer, NSCLC, and melanoma are shown in green. Number of drugs that are common in two cancer types are shown in pink and common in three cancer types shown in purple. Drugs able to cross BBB are shown in yellow. Five drugs that can cross BBB are shared between breast cancer and NSCLC patients, while three are common between NSCLC and melanoma patients. There are no common drugs between three cancer groups that can penetrate BBB. BBB, blood-brain barrier; NSCLC, non-small cell lung cancer.

Articles included in the current study

Study	Author	Primary Tumor Location
Recurrently Mutated Genes Differ between Leptomeningeal and Solid Lung Cancer Brain Metastases	Li Y et al. 2018	NSCLC
Detection of Driver and Resistance Mutations in Leptomeningeal Metastases of NSCLC by Next-Generation Sequencing of Cerebrospinal Fluid Circulating Tumor Cells	Jiang et al. 2017	NSCLC
Unique genetic profiles from cerebrospinal fluid cell-free DNA in leptomeningeal metastases of EGFR-mutant non-small-cell lung cancer: a new medium of liquid biopsy	Li YS et al. 2018	NSCLC
Unique genomic profiles obtained from cerebrospinal fluid cell-free DNA of non-small cell lung cancer patients with leptomeningeal metastases	Ying et al. 2019	NSCLC
Unique Genomic Alterations of Cerebrospinal Fluid Cell-Free DNA Are Critical for Targeted Therapy of Non-Small Cell Lung Cancer with Leptomeningeal Metastasis	Wang et al. 2021	NSCLC
Circulating tumor cell characterization of lung cancer brain metastases in the cerebrospinal fluid through single-cell transcriptome analysis	Ruan et al. 2020	NSCLC
Different next-generation sequencing pipelines based detection of tumor DNA in cerebrospinal fluid of lung adenocarcinoma cancer patients with leptomeningeal metastases	Ge et al. 2019	NSCLC
Detection of circulating tumor DNA from non-small cell lung cancer brain metastasis in cerebrospinal fluid samples.	Ma et al. 2020	NSCLC
Clinical Utility of Cerebrospinal Fluid Cell-Free DNA as Liquid Biopsy for Leptomeningeal Metastases in ALK-Rearranged NSCLC	Zheng et al. 2019	NSCLC
Brain tumor mutations detected in cerebral spinal fluid.	Pan et al. 2015	NSCLC
Cell-Cycle and DNA-Damage Response Pathway Is Involved in Leptomeningeal Metastasis of Non–Small Cell Lung Cancer	Fan et al. 2018	NSCLC
Genotyping tumour DNA in cerebrospinal fluid and plasma of a HER2-positive breast cancer patient with brain metastases	Siravegna et al. 2017	Breast cancer
Clinical significance of detecting CSF-derived tumor cells in breast cancer patients with leptomeningeal metastasis	Li X et al. 2017	Breast cancer
Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma	De Mattos-Arruda et al. 2015	Breast cancer
Tumor DNA in cerebral spinal fluid reflects clinical course in a patient with melanoma leptomeningeal brain metastases	Li Y et al. 2016	Melanoma
Evaluating Circulating Tumor DNA From the Cerebrospinal Fluid of Patients with Melanoma and Leptomeningeal Disease	Ballester et al. 2018	Melanoma

Drug candidates that can cross BBB for LMC treatment

Drug Candidate	Target Gene	Clinical Use	Clinical Trials
Everolimus	*PTEN, PIK3CA*	NSCLC Breast cancer	Melanoma
Bevacizumab	*PIK3CA, TP53*	NSCLC	NSCLC-LMC Breast-LMC Melanoma
Temozolomide	*PTEN, TP53*	-	Breast-LMC NSCLC Melanoma