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A Transcriptome- and Interactome-Based Analysis Identifies Repurposable Drugs for Human Breast Cancer Subtypes

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Abstract: Breast cancer (BC) is a heterogeneous and complex disease characterized by different subtypes with distinct morphologies and clinical implications and for which new and effective treatment options are urgently demanded. The computational approaches recently developed for drug repurposing provide a very promising opportunity to offer tools that efficiently screen potential novel medical indications for various drugs that are already approved and used in clinical practice. Here, we started with disease-associated genes that were identified through a transcriptome-based analysis, which we used to predict potential repurposable drugs for various breast cancer subtypes by using an algorithm that we developed for drug repurposing called SAveRUNNER. Our findings were also in silico validated by performing a gene set enrichment analysis, which confirmed that most of the predicted repurposable drugs may have a potential treatment effect against breast cancer pathophenotypes.

Keywords: breast cancer subtypes; switch genes; drug repurposing; SAveRUNNER

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1. Introduction

Breast cancer (BC) is a major public health problem that impacts more than two million women worldwide each year [1]. BC is characterized by a heterogeneous nature at the histological, molecular, and systemic levels, as witnessed by the existence of different subtypes with distinct morphologies and clinical implications [2]. A BC subtype classification can be based on clinical parameters or histopathologic markers, such as the presence/absence of ER-estrogen receptors, PR-progesterone receptors, and HER2-human epidermal growth factor receptors [3]; or on gene expression values of specific molecular markers, such as in PAM50 classification, which recognizes four intrinsic subtypes, i.e., luminal A, luminal B, Her2-enriched, and basal-like [4]. Although the PAM50 classification offers more accurate clinical information than the classifications based on histopathologic parameters [3], the management and effective treatment of these different pathophenotypes still remains a challenge for clinicians who often have to resort to highly unspecific cytotoxic therapies.

To address this challenge, several theoretical and methodological advances have been proposed in recent years that aim to develop improved therapeutic options. These advances include a new field of medicine called Network Medicine, which applies tools and concepts from network theory to elucidate the relation between perturbations on the molecular-level and phenotypic disease manifestations [5,6]. According to this revolutionary idea, diseases are rarely caused by the deregulation of a single gene, but more typically they are the result of molecules associated with a given disease (i.e.,

disease genes) and co-localized in specific regions (i.e., disease module) of the human interactome (i.e., the integrated network of all physical interactions within the cell) [5,6]. The Network Medicine paradigm states that not only a given disease but also the action of a given drug can be interpreted as a perturbation within the human interactome. As a consequence, for a drug to be on-target effective against a specific disease or to cause off-target adverse effects, its targets should be within or in the immediate vicinity of the corresponding disease module in the interactome [6]. This construct has fueled the development of several computational approaches for detecting novel therapeutic targets as well as drug repurposing candidates [7–10]. Drug repurposing (DR) is defined as the process whereby an existing drug (already FDA-approved or in the trial/experimental phase) is used for the treatment of a disease other than its primary or initial purpose, which represents an effective alternative strategy to the very time-consuming and costly process of de novo drug discovery [11,12]. One the most promising algorithms that has recently been developed for drug repurposing is SAveRUNNER (Searching off-lAbel dRUg aNd NEtwoRk) [10,13], which offers an interactome-based tool that efficiently screens potential novel medical indications for currently marketed drugs against diseases of interest.

In the present study, we used SAveRUNNER to identify putative repurposable drugs for the breast cancer subtypes defined by the PAM50 classification. Indeed, specific and consolidated drugs that treat the four PAM50 BC subtypes are still not available [14]. As input, SAveRUNNER requires a list of genes associated with a particular disease (disease genes). Since knowledge of the genes associated with each PAM50 breast cancer subtype is not currently well-established, here we used as disease genes the results of our previous study [15], in which we studied the transcriptomic data of TCGA (The Cancer Genome Atlas) patients affected by breast invasive carcinoma and stratified them according to the PAM50 classification. In [15], we identified a list of genes that are likely associated with each BC subtype by using SWIM (SWItch Miner), a network-based methodology recently developed by our group that gained broad approval in recent years thanks to its successful application in the field of Network Medicine [16,17].

Overall, the integrated analysis presented here has led to the in silico identification of potential repurposable drugs for the various BC subtypes and paves the way for further investigation and subsequent experimental validation.

2. Materials and Methods

2.1. Network-Based Tools

2.1.1. SWIM

SWIM (SWItch Miner) is a freely downloadable network-based tool, developed both in MATLAB [16] and in the R language [17], which predicts important (switch) genes that are strongly associated with drastic changes in cell phenotype. A fully comprehensive description of SWIM's methodology is provided in [16,17]. Briefly, SWIM first computes the differentially expressed genes (DEGs) between two conditions of interest (e.g., normal state versus tumour state) and then builds gene expression networks (GENs) by calculating correlations (positive and negative) between the expression profiles of each gene pair. Next, it identifies a special set of genes (called switch genes) within GENs that are associated with intriguing patterns of molecular co-abundance and may play a key role in phenotypic transitions in various biological settings [16,18–20]. Switch genes are characterized by peculiar topological features that can be summarized as follows: (i) they show coherent patterns of correlation, suggesting they may be co-regulated or functionally related; (ii) they form localized connected subnetworks/modules in the correlation network; (iii) they are not local hubs within their module, but they act as connectors that can convey information among the modules of the correlation network.

2.1.2. SAveRUNNER

SAveRUNNER (Searching off-lAbel dRUg aNd NEtwoRk) is a freely downloadable network-based tool, developed in R language [10,13], which generates predictions of repurposable drugs against diseases of interest, thus improving the discovery rate of new therapeutic modalities. A comprehensive description of SAveRUNNER's methodology is provided in [10,13]. The hypothesis underlying SAveRUNNER's methodology is that for a drug to be effective against a disease, its associated targets (drug module) and disease-associated genes (disease module) should be topologically close to each other in the human interactome [8]. To quantify the vicinity between drug and disease modules, SAveRUNNER implements a novel network-based similarity measure (called adjusted similarity) that prioritizes associations among drugs and diseases located in the same network neighborhoods [10,13]. In particular, SAveRUNNER first computes the network-based proximity between the drug and disease modules and evaluates its statistical significance by applying a degree-preserving randomization procedure. Next, it translates the proximity measure into a similarity measure and leverages it to apply a clustering algorithm that groups similar drugs and diseases. Finally, it exploits the results of the clustering analysis to adjust the network similarity and to reward associations among drugs and diseases belonging to the same cluster, based on the assumption that if a drug and a disease group together it is more likely that the drug can be effectively repurposed for that disease [10,13]. SAveRUNNER provides a list of predicted/prioritized associations among drugs and diseases in the form of a weighted bipartite drug–disease network, where one set of nodes represents drugs and the other represents diseases. A link between a drug and a disease is made if the corresponding drug targets and disease genes are closer in the interactome than is expected by chance, with an interaction weight based on the adjusted similarity value [10].

2.2. Data Retrieval

A transcriptome-based analysis was conducted in our previous study [15], in which we applied the SWIM methodology to the gene expression data of TCGA breast invasive carcinoma (BRCA) affected patients, stratified according to the PAM50 genetic classification [4,21]. A summary of the results obtained in [15] is reported in Table 1.

Table 1. Summary of SWIM analysis for the breast cancer subtypes of PAM50 classification. Symbol # is an abbreviation for number.

	Luminal A	**Luminal B**	**HER2 Enriched**	**Basal-Like**
Samples				
# normal	111	111	111	111
# tumour	229	120	58	98
switch genes				
# total	222	358	363	343

Here, we used the list of switch genes obtained in [15] as disease genes that we inputted into SAveRUNNER in order to predict novel putative repurposable drugs for treating the different PAM50 breast cancer subtypes. A detailed description of all input data required by SAveRUNNER is provided in Table 2.

Table 2. Input data for the interactome-based analysis.

Input	Source	Publication Year/Version	Description
PAM50 switch genes	Grimaldi et al. [15]	2020	see Table 1
Human interactome	Cheng et al. [8]	2018	217,160 physical interactions connecting 15,970 biomolecules
Drug–target associations	DrugBank [22]	version 5.1.6 released on 22 April 2020	2165 genes interacting with 1873 drugs
Drug original indications	Therapeutic Target Database (TTD) [23]	version released on 1 June 2020	5059 drugs associated with 1136 diseases

2.3. In Silico Validatio: GSEA Analysis

To test whether the repurposable drugs predicted by SAveRUNNER could counteract the gene expression perturbations caused by the breast cancer subtypes (i.e., can up-regulate genes down-regulated by the disease or vice versa), we performed a gene set enrichment analysis (GSEA) through the Connectivity Map (CMap) query tool [23]. This analysis may represent an in silico validation and requires input of a disease signature and a drug signature, which were: (i) as the disease signature, we used the switch genes that were computed by SWIM for each subtype of the PAM50 classification (Supplementary Table S1); (ii) as the drug signature, we used the differentially expressed genes of drug-treated human breast cancer cell lines provided by the CMap database [23,24]. Indeed, the CMap database collects transcriptional responses of a variety of human cells to chemical and genetic perturbation (perturbagen) [23,24]. In our analysis, we selected as perturbagen types only the small-molecule compounds (i.e., trt_cp), and as cell lines (if available) the breast cancer cell lines conventionally used as models for a specific BC subtype (i.e., MCF7 for luminal A; SKBR3 for HER2-enriched; and BT20, MDAMB231, and HS578T for basal-like). Concerning luminal B, a representative cell line is lacking in the CMap database and thus we considered the MCF7 cell line associated with the other luminal subtype, luminal A.

For each drug that was both included in the CMap database and predicted by SAveRUNNER, we extracted the so-called enrichment score (ES) from the Cmap query tool, which evaluates whether the effect of the drug could counteract the effect of the disease ($ES < 0$) or not ($ES > 0$) [24–26]. Specifically, the computation of the ES requires that both the differentially expressed genes of the disease signature and the differentially expressed genes of the drug signature are ordered by increasing fold-change. Then, these two lists of differentially expressed genes are compared for determining if the highest up-regulated (down-regulated) genes of the disease signature are near the bottom (top) of the drug signature. In other words, a candidate repurposable drug was considered to have a potential treatment effect against a given disease if the drug signature was negatively correlated with the tested disease signature ($ES < 0$). Finally, for each tested drug, we either assigned a GSEA value equal to 1 if that drug was found to have $ES < 0$ with respect to a given disease or a GSEA value of 0 if otherwise.

3. Results

3.1. Study Design

In the present study, we aimed to predict effective repurposable drugs for treating the different molecular BC subtypes of PAM50 classification by integrating the outcomes of two recent and promising tools of Network Medicine: the transcriptome-based SWIM tool [16,17], which is able to identify putative disease-associate genes (called switch genes); and the interactome-based SAveRUNNER tool [10,13], which is able to identify putative off-label drugs to be repurposed. We complemented our integrated pipeline

with an in silico validation of the candidate repurposable drugs using a gene set enrichment analysis (GSEA) [13,26,27]. An overview of our study is depicted in Figure 1.

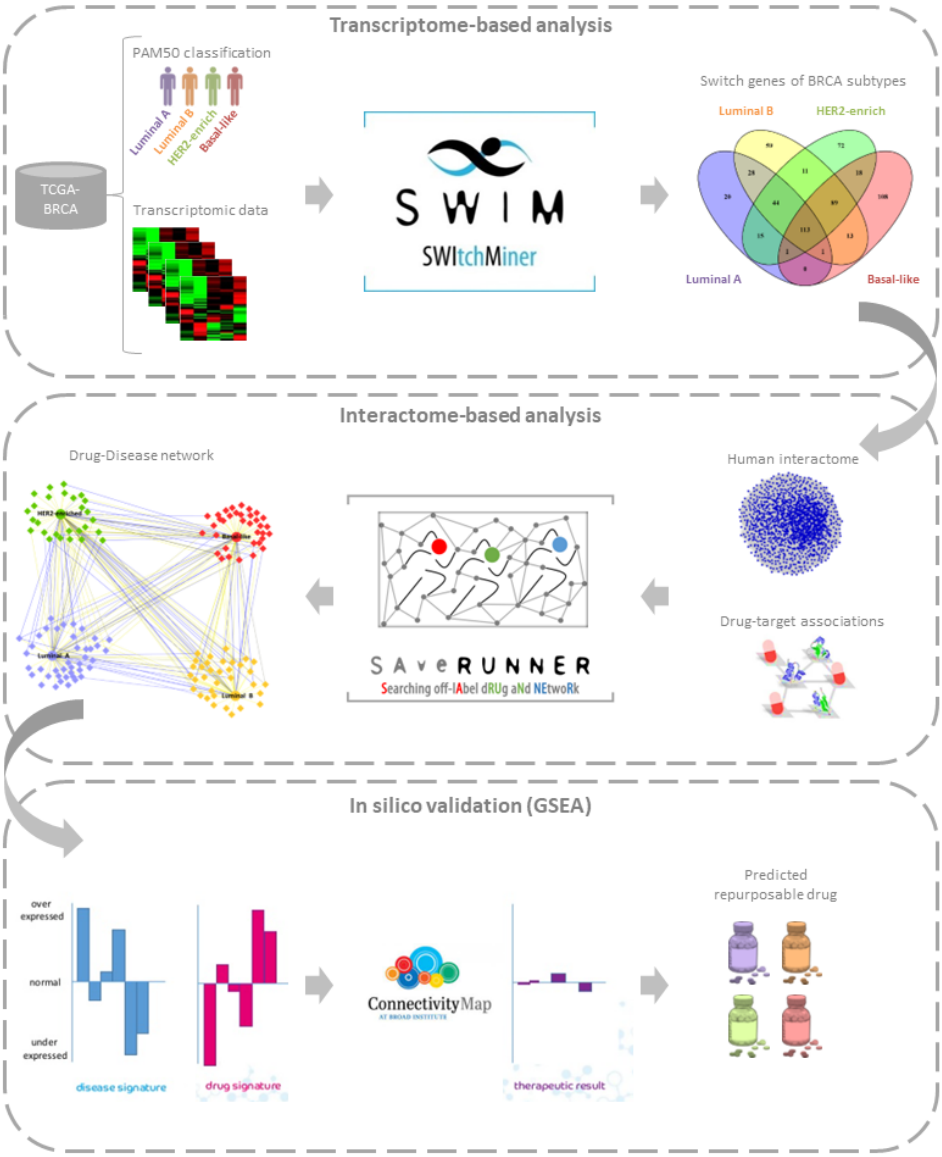

Figure 1. Study overview. Transcriptome- and interactome-based analyses were exploited to identify repurposable drugs for breast cancer subtypes. The results were in silico validated by a gene set enrichment analysis (GSEA).

3.2. Transcriptome-Based Analysis

To date, the successful broad application of SWIM has contributed to depict switch genes as key regulators of the phenotypic transitions in various biological settings [16,18–20,28]. In our previous study [15], we applied the SWIM tool to the transcriptomic data of 505 TCGA-BRCA patients stratified according to the well-consolidated PAM50 classification (229 subjects luminal A, 120 subjects luminal B, 58 subjects HER2-enriched, and 98 subjects basal-like) to identify switch genes associated with each BC subtype. We found that they are mostly upregulated in the tumour condition (Supplementary Table S1) and form statistically significant overlapping modules in the human interactome [15].

3.3. Interactome-Based Analysis

The switch genes identified for the four molecular BC subtypes of the PAM50 classification were used as disease genes to input into SAveRUNNER together with the drug targets of 1873 FDA-approved drugs provided by DrugBank [22] (Table 2). Disease genes and drug targets were mapped by SAveRUNNER on the human interactome and assembled by Cheng and coauthors in [8] (Table 2). In the present study, we obtained a drug–disease network of a total of 141 nodes (i.e., 4 BC subtypes and 137 drugs) that were connected by 284 edges and grouped into 4 well-separated clusters (Figure 2).

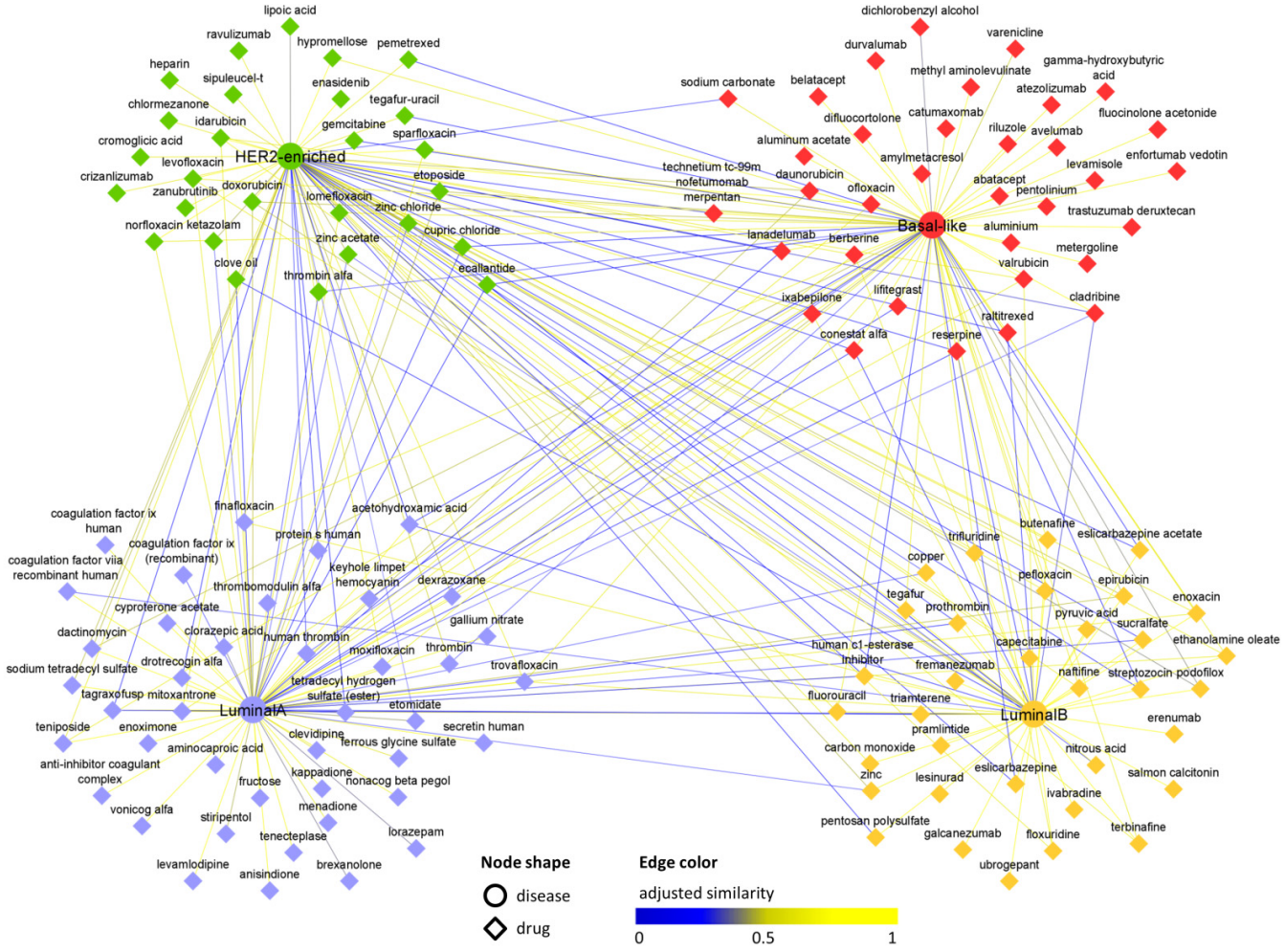

Figure 2. Drug–disease network predicted by SAveRUNNER. Nodes refer to the four BC subtypes (circles) and the FDA-approved drugs (diamonds). Nodes are colored according to the clusters identified by SAveRUNNER. The edges refer to the predicted drug–disease association by SAveRUNNER. Edges are colored according to the adjusted similarity measure,increasing from blue (less similar) to yellow (more similar).

SAveRUNNER predicted 74 repurposable drugs for luminal A, 54 repurposable drugs for luminal B, 79 repurposable drugs for HER2-enriched, and 77 repurposable drugs for basal-like (Supplementary Table S2). Among them, 18 were found to be shared among all subtypes, 23 specific to luminal A, 9 specific to luminal B, 9 specific to HER2-enriched, and 22 specific to basal-like (Figure 3a and Supplementary Table S2).

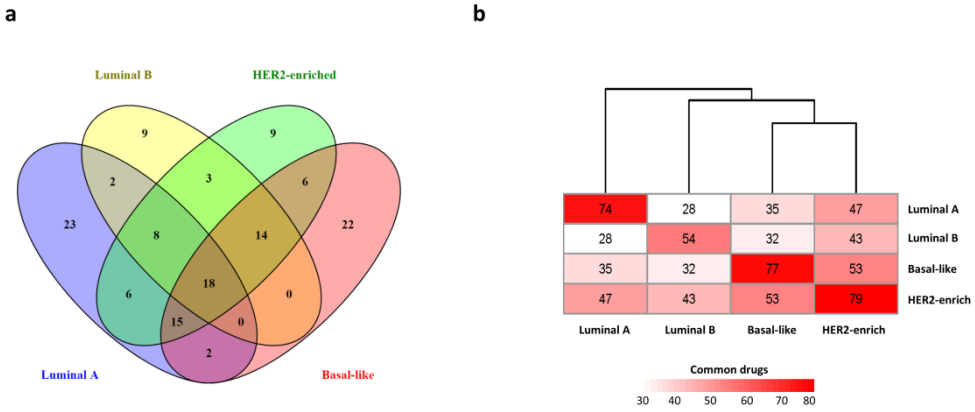

Figure 3. Repurposable drugs of BC subtypes. (**a**) Venn diagram detailing the counts of specific and common repurposable drugs identified by SAveRUNNER among the four BC subtypes of PAM50 classification. (**b**) Comparative analysis of the repurposable drugs in the four BC subtypes. The distance matrix, computed based on Hamming distance of binary-encoded (1 = present, 0 = absent) annotation of repurposable drugs across the four BC subtypes, is rendered in a symmetrical heat map where each cell reports the counts (increasing from white to red scale) of drugs shared between subtype pairs. Dendogram on columns of this heatmap indicate subtype clustering based on Hamming distance.

To compare the four BC subtypes in terms of predicted drugs, SAveRUNNER translated the drug–disease network into a binary matrix by assigning a 1 if a given drug (matrix row) was found to be repurposable for a given BC subtype (matrix column) and a 0 if otherwise. Then, the Hamming distance was used to group BC subtypes based on the similarity of the repurposable drugs (Figure 3b). The results of this analysis pointed out how the two aggressive subtypes (i.e., HER2-enriched and basal-like) are more similar in terms of putative drug repurposing treatments while the less aggressive subtype (i.e., luminal A) represents an outlier.

We also investigated the original medical indications of the SAveRUNNER-predicted drugs and found high heterogeneity with drugs approved to treat other tumors, infections, hypertension, etc. In particular, we found that 20% (28/137) of the total predicted drugs have an indication originally related to cancer treatment, including three drugs (i.e., *dexrazoxane*, *ixabepilone*, *capecitabine*) already approved for fighting breast cancers (Supplementary Table S2).

3.4. In Silico Validation

In order to in silico validate the repurposable drugs predicted by SAveRUNNER, we performed a gene set enrichment analysis with the CMap query tool [23]. For each BC subtype, we used the switch genes identified by SWIM-based analysis as the disease signature; whereas we used information about a drug's effect on human breast cancer cell lines available in the CMap database as the drug signature (see Section 2). Using these inputs, the CMap query tool computed a score, i.e., the enrichment score (ES), which provided an indication of the possible counteraction by each drug of the gene expression perturbations caused by each breast cancer pathophenotype. Next, we selected drugs whose signatures were negatively correlated with the disease signature of each BC subtype (ES < 0) as drugs potentially able to be effective against gene products that are putative hallmarks of that BC subtype (GSEA = 1 in the Supplementary Table S2).

The GSEA analysis highlighted a total of 21 candidate repurposable drugs for luminal A, 20 for luminal B, 1 for HER2-enriched, and 8 for basal-like (Figure 4 and Supplementary Table S2).

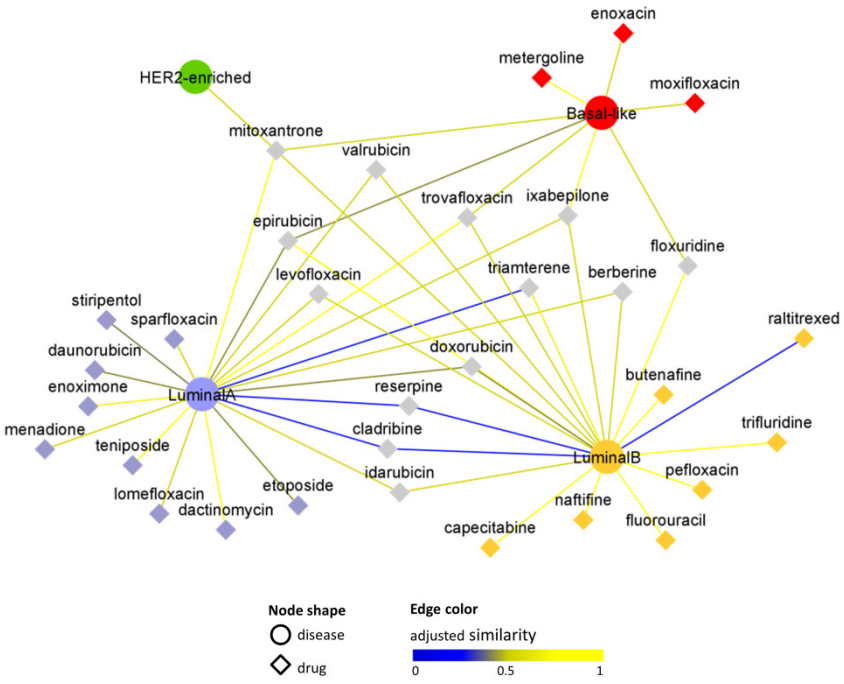

Figure 4. Drug–disease network filtered by the in silico validation. This bipartite network shows the SAveRUNNER predicted drug–disease associations connecting the four BC subtypes (circles) with the FDA-approved drugs (diamonds) after the GSEA analysis. The edge color refers to the adjusted similarity between drug targets and disease genes mapped on the human interactome, increasing from blue (less similar) to yellow (more similar). Drugs predicted to be specifically repurposed for a given BC subtype are coloured according to the label colour of that BC subtype, whereas drugs predicted to be repurposed for more than one BC subtypes are coloured in grey.

4. Discussion

The network medicine-based approach for drug repurposing has matured considerably in recent years, now possessing both a firm theoretical fundament, as well as a wide range of successful applications across various human diseases [8–10,27,29,30]. Among them, one of the most promising is SAveRUNNER, a tool we recently developed to predict potential novel uses of already approved drugs by quantifying the interplay between the drug targets and the disease-associated genes in the human interactome [13]. In the present study, we leveraged SAveRUNNER to screen drugs that can be repurposed against different breast cancer subtypes for which specific and effective treatment strategies do not yet exist. In particular, we considered the four molecular BC subtypes of the well-established PAM50 classification, which are (from the less aggressive to the more aggressive) luminal A, luminal B, HER2-enriched, and basal-like. As disease-associated genes for these four BC subtypes, we exploited the (switch) genes previously identified in [15] through the transcriptome-based analysis implemented by the SWIM tool. Indeed, we recently demonstrated that switch genes satisfy all of the hypotheses and organizing principles formalized by the network medicine construct, in the same way as disease genes themselves do, and thus they can reasonably be considered as novel candidate disease genes for a given disease of interest [28].

At the end of our transcriptome- and interactome-based analysis, we identified a total of 137 unique drugs to be repurposed against the four PAM50 BC subtypes. In particular, we predicted 74 repurposable drugs for luminal A, 54 repurposable drugs for luminal B, 79 repurposable drugs for HER2-enriched, and 77 repurposable drugs for basal-like (Figure 3a and Supplementary Table S2). Next, these candidate repurposable drugs were also in silico validated by performing a GSEA analysis (Figure 4 and Supplementary Table S2).

Among the drugs predicted for the luminal A subtype with the highest adjusted similarity value (adjusted similarity ~ 1), we found *finafloxacin*, *moxifloxacin*, *mitoxantrone, teniposide,* and *dactinomycin*. Notably, the potential treatment effect against luminal A of the last three cited drugs was also corroborated by GSEA analysis (Figure 4). Both *finafloxacin* and *moxifloxacin* are fluoroquinolone antibiotics used to treat various bacterial infections. Recent studies have confirmed that bacterial infections are an important contributor in cancer and elimination of tumor-associated microbes may lead to a reduction in tumors and improved survival [31–34]. Thus, the repositioning of fluoroquinolones into an anticancer molecule seems to be a highly plausible option [35]. Concerning the chemotherapeutic agent *mitoxantrone*, a very recent study [36] showed that its combination with rapalogs (i.e., analogs of rapamycin) can exert a highly synergistic antitumor effect in breast cancer cells by blocking the eEF-2K-mediated activation of Akt, both in vitro and in vivo. *Teniposide* is a cytotoxic drug used for the treatment of refractory acute lympho-blastic leukaemia. A recent study by Chu et al. [37] demonstrated that the intravenous application of *teniposide* suppresses the growth of subcutaneous MCF-7 (luminal A cell line) in vivo, thus exhibiting a strong anticancer effect. *Dactinomycin* is currently used to treat a wide variety of cancers and it has been proposed as a potential anti-breast cancer agent. Indeed, Das et al. showed that this compound is able to induce death in breast cancer stem cells by downregulating SOX2 expression [38]. Other interesting antiluminal A drugs that were predicted by SAveRUNNER and validated by GSEA are *menadione* (adjusted similarity = 0.87), *daunorubicin* (adjusted similarity = 0.65), *etoposide* (adjusted similarity = 0.65), and *stiripentol* (adjusted similarity = 0.63). In particular, *menadione* is a fat-soluble vitamin precursor mainly used to treat vitamin K deficiency and prostate cancer. Interestingly, Marchionatti et al. [39] demonstrated that *menadione* has an antiproliferative effect on breast cancer MCF-7 cells and more recently Guizzardi et al. [40] showed that its combination with calcitriol may increase the antiproliferative effect by promoting oxidative/nitrosative stress, mitochondrial dysfunction, and autophagy. *Daunorubicin* is currently indicated for inducing remission of nonlymphocytic leukemia and acute lymphocytic leukemia. However, Zhang et al. proved that the use of *daunorubicin* in combination with *quinacrine* may be an effective treatment for MCF-7 cancer cells in vitro, MCF-7 cancer stem cells in vitro, and relapsed tumors in mice [41]. Concerning *etoposide*, it is used in combination with other chemotherapeutic agents for treating various malignancies such as testicular tumors, lymphoma, non-lymphocytic leukemia, and glioblastoma multiforme. Several studies proposed *etoposide* as a valuable and safe option for pre-treated metastatic breast cancer patients [42–45]. Finally, *stiripentol* is an antiepileptic agent used in combination with other anticonvulsants to treat seizures associated with Dravet syndrome. Interestingly, Bonucelli et al. [46] conducted metabolic in vitro experiments on breast cancer MCF-7 cells and identified *stiripentol* as a potential therapeutic option that is able to effectively inhibit the propagation of cancer stem-like cells.

The top-ranked drugs predicted by SAveRUNNER for luminal B subtype (adjusted similarity ~ 1) include *ivabradine*, *capecitabine*, and *fluorouracil*. *Ivabradine* is a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker used for the symptomatic management of stable angina pectoralis and symptomatic chronic heart failure. This compound was recently repurposed as a novel therapy for breast cancer by Mok et al. [47]. Indeed, the authors of this study found that HCN inhibition by *ivabradine* is able to block breast cancer cell proliferation in vitro and to suppress tumour growth in patient-derived tumour xenograft models. *Capecitabine* and *fluorouracil*, both validated by GSEA analysis (Figure 4), are orally-administered chemotherapeutic agents that are already in use for the treatment of metastatic breast cancer [48,49].

Among the most promising repurposable drugs that were proposed for treating HER2-enriched subtype (adjusted similarity ~ 1), we found several compounds indicated for the treatment of bacterial infections, i.e., *lomefloxacin*, *levofloxacin*, *ofloxacin*,

norfloxacin, and *sparfloxacin* (Supplementary Table S2). We would highlight that the only candidate repurposable drug remaining downstream of the GSEA analysis for the treatment of HER2-enriched subtype (Figure 4) is the above-discussed *mitoxantrone* (adjusted similarity = 0.87).

Finally, among the top repurposable drugs that our pipeline identified to treat the most aggressive BC subtype, i.e., basal-like, was *metergoline* (adjusted similarity ~ 1). *Metergoline* is an ergot-derivative acting as antagonist of certain 5-HT receptor subtypes and as agonist of dopamine receptors. It is often indicated when an inhibition of prolactin is desirable such as premenstrual dysphoric disorder in women and antianxiety treatment. However, a very recent study demonstrated that 5-HT induces cell proliferation of triple-negative breast cancer (genetically and clinically similar to basal-like phenotype [50]) through 5-HT7 receptor signaling [51]. Thus, the genetic and pharmacological inhibition of 5-HT7 by RNAi and *metergoline*, respectively, may lead to suppression of triple-negative breast cancer cell proliferation. The putative treatment effect of *metergoline* against the basal-like subtype was also in silico validated (GSEA = 1). Another interesting SAveRUNNER-predicted drug with high adjusted similarity for basal-like is *riluzole* (adjusted similarity = 0.93). Indeed, it is a glutamate antagonist used to treat amyotrophic lateral sclerosis that was recently also evaluated in cancer cells [52]. Specifically, the results of this study indicate that *riluzole* is able to arrest cell proliferation as well as induce cell death in cancers of various tissue origins, including breast, pancreas, liver, bone, brain, and lung [52]. Moreover, *riluzole* was found to mediate antitumor properties in triple-negative breast cancer cells independently of metabotropic glutamate receptor-1 [53–55].

5. Conclusions

The present study showed how a transcriptome- and interactome-based analysis, exploiting tools and concepts of the Network Medicine paradigm, may contribute to the prediction of novel repurposable drugs for fighting the heterogeneity of breast cancer phenotype. We are aware that our study has a purely computational nature and experimental validations are mandatory to prove the actual efficacy of the predicted drugs in the treatment of the different BC subtypes. However, we believe that our findings could pave the way for the discovery of hidden off-label uses of already approved drugs, maximizing the efficiency of the downstream validation experiments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/sym14112230/s1. Supplementary Table S1: SWIM results. The file is composed of four separate sheets that list the switch genes computed by SWIM and their statistics, for each of the four BC subtypes analysed in the study. Supplementary Table S2: SAveRUNNER results. The file is composed of seven separate sheets. The first four sheets list the repurposable drugs predicted by SAveRUNNER and their statistics, for each of the four BC subtypes analysed in the study. The fifth sheet lists the repurposable drugs predicted by SAveRUNNER with their known medical indications. The sixth sheet lists the specific and common repurposable drugs predicted by SAveRUNNER for the four BC subtypes analysed in the study. The seventh sheet lists the SAveRUNNER-predicted drugs remaining after the in silico validation (GSEA).

Author Contributions: P.P.: conceptualization, supervision, project administration, funding acquisition, software development; F.C.: methodology, formal analysis, software development, writing–original draft; P.S.: validation, formal analysis; G.F.: data interpretation, software development. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Gene expression data used in our previous study [15] are freely available from the TCGA repository (TCGA-BRCA project). The human interactome was retrieved from the supplementary files (i.e., Supplementary Data S1) of [8]. Drug–target associations were retrieved from DrugBank database [22]. Drug medical indications were retrieved from Therapeutic Target Database (TTD) [56]. SWIM tool is freely available on GitHub at the following link: https://github.com/sportingCode/SWIMmeR (accessed on 1 January 2022). SAveRUNNER tool is freely available on GitHub at the following link: https://github.com/sportingCode/SAveRUNNER (accessed on 1 February 2022). All the other relevant data are within this manuscript and its Supporting Information files.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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