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Cellular Fitness Phenotypes of Cancer Target Genes from Oncobiology to Cancer Therapeutics

Bijesh George^{1,2}, P. Mukundan Pillai^{1,†}, Aswathy Mary Paul^{1,2,†}, Revikumar Amjesh¹, Kim Leitzel³, Suhail M. Ali⁴, Oleta Sandiford⁵, Allan Lipton³, Pranela Rameshwar⁵, Gabriel N. Hortobagyi⁶, Madhavan Radhakrishna Pillai^{1,*} and Rakesh Kumar^{1,4,7,*}

- ¹ Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala 695014, India; bijeshgeorge@rgcb.res.in (B.G.); mukundan@rgcb.res.in (P.M.P.); aswathym@rgcb.res.in (A.M.P.); amjeshr@rgcb.res.in (R.A.)
- ² Graduate Degree Program, Manipal Academy of Higher Education, Manipal 576104, India
- ³ Hematology-Oncology, Department of Medicine, Penn State University School of Medicine, Hershey, PA 17033, USA; kleitzel@pennstatehealth.psu.edu (K.L.); alipton@pennstatehealth.psu.edu (A.L.)
- ⁴ Lebanon Veterans Affairs Medical Center, Lebanon, PA 17042, USA; Suhail.Ali@med.va.gov
- ⁵ Department of Medicine, Division of Hematology & Oncology, Rutgers New Jersey Medical School, Newark, NJ 07103, USA; oas26@njms.rutgers.edu (O.S.); Rameshwa@njms.rutgers.edu (P.R.)
- ⁶ Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Centre, Houston, TX 77030, USA; ghortoba@mdanderson.org
- ⁷ Department of Human and Molecular Genetics, Virginia Commonwealth University Medical Center, Richmond, VA 23298, USA
- * Correspondence: rakeshkumar@rgcb.res.in (R.K.); mrpillai@rgcb.res.in (M.R.P.)
- † Equal contribution.



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Abstract: To define the growing significance of cellular targets and/or effectors of cancer drugs, we examined the fitness dependency of cellular targets and effectors of cancer drug targets across human cancer cells from 19 cancer types. We observed that the deletion of 35 out of 47 cellular effectors and/or targets of oncology drugs did not result in the expected loss of cell fitness in appropriate cancer types for which drugs targeting or utilizing these molecules for their actions were approved. Additionally, our analysis recognized 43 cellular molecules as fitness genes in several cancer types in which these drugs were not approved, and thus, providing clues for repurposing certain approved oncology drugs in such cancer types. For example, we found a widespread upregulation and fitness dependency of several components of the mevalonate and purine biosynthesis pathways (currently targeted by bisphosphonates, statins, and pemetrexed in certain cancers) and an association between the overexpression of these molecules and reduction in the overall survival duration of patients with breast and other hard-to-treat cancers, for which such drugs are not approved. In brief, the present analysis raised cautions about off-target and undesirable effects of certain oncology drugs in a subset of cancers where the intended cellular effectors of drug might not be good fitness genes and that this study offers a potential rationale for repurposing certain approved oncology drugs for targeted therapeutics in additional cancer types.

Keywords: cancer fitness genes; breast cancer hard-to-treat cancers; Mevalonate and Purine biosynthesis; oncology drugs; repurposing

1. Introduction

Over the past few decades, cancer treatment has witnessed tremendous progress in disease-free survival and in the delay and/or prevention of cancer recurrence in cancer patients. The first-generation of cancer chemotherapeutics and cytotoxic drugs generally target cellular processes that are fundamental to the growth of both cancer and normal cells, such as nucleic acids, protein synthesis and cell metabolism. Such drugs exhibit both anticancer and toxic side effects due to their non-specificity with respect to targets in

cancer and normal cells in addition to other variables of drug metabolism etc. [1–4]. By contrast, a targeted cancer therapy targets specific cellular biomolecules and pathway(s) that are differentially overexpressed and/or hyperactivated in cancer cells as compared with normal cells and has emerged as a preferred option in the treatment of cancer [5–7]. The US Food and Drug Administration (FDA) has approved approximately 235 oncology drugs until May 2019, which target or utilize approximately 232 cellular components. The core of targeted cancer therapy is the intended cellular target against which an inhibitory molecule was developed. However, targeted cancer therapeutics could also result in both beneficial and toxic effects due to the on- and off-target effects of the drug [2,8,9]. The mere presence of an upregulated cellular molecule or target in cancer cells and its activity does not ensure that a given cancer drug will exhibit a homogenous therapeutic response across the patient population with a given cancer subtype. For example, despite a widespread overexpression of human epidermal growth factor receptor 2 (HER2) in breast cancer patients, only approximately 26% of patients with breast cancer receiving trastuzumab (an anti-HER2 monoclonal antibody) as a single agent exhibited a beneficial clinical response [10], whereas approximately 34% of patients with metastatic colorectal cancer positive for epidermal growth factor receptor (EGFR) presented stable disease upon receiving cetuximab (an anti-EGFR monoclonal antibody) as a single agent. Thus, a majority of cancer patients receiving monotherapy exhibited the progression of cancer despite of the presence of its intended target—generally the basis for cancer patient’s enrolment for a given targeted therapy [11]. HER2-directed therapies such as trastuzumab therapy in patients with breast cancer resulted in a median survival of over 3 years [12], whereas the therapy led to a modest increase by approximately 4 months in the median survival of patients with gastric cancer [13]. Such somewhat limited beneficial effects could be due to the inherent genomic and cellular heterogeneity, acquired compensatory rewiring of proliferative and survival pathways [10–14], or unidentified mechanism of drug action, etc. Moreover, whether the differential effectiveness of targeted therapy in these settings was due to the lack of or accessibility to the intended target or due to the ineffectiveness of the targeted therapy in inhibition of the target [8,9,14] remains unclear.

Currently available FDA-approved oncology drugs have been developed through molecule-driven empirical approaches [15]. This has also been very fruitful and was somewhat essential to reach the current stage of targeted cancer therapy. However, these approaches did not fully consider the post-genomic data or the fact that cancer is a polygenic disease in selecting the target for developing a drug [16]. Although the polygenic nature of cancer [17] was not always factored during the development of FDA-approved oncology drugs, it is generally considered in the development of combination therapy. Additionally, the post-genomic data and high-throughput screening platforms are actively utilized for the molecular classification and diagnosis of tumors, assessment of the therapeutic sensitivity, and patient stratification to improve the effectiveness of existing oncology drugs [18–20].

Targeted cancer therapy is still unable to inhibit the growth of all tumor cells of a given cancer-type in patients. It is possible that a new approach might be helpful for additional benefits for cancer patients. In this context, Behan et al. developed a comprehensive portrait of the gene dependency of human cancer [21] wherein the team utilized the CRISPR-Cas9 approach to selectively knock out approximately 7460 genes in 324 well-characterized cell lines with respect to their genomes [22]. The team assayed the requirement of each gene for the cellular fitness (viability) of cancer cell lines representing 19 cancer-types. The results were depicted as a negative fitness effect (the loss of cell viability in the absence of a test gene) or positive fitness effect (no loss of cell viability in the absence of a test gene), with the outcome presented as “fitness gene” or “not a fitness gene” for each gene in 324 cell lines [21,23]. The work by Behan et al. identified 628 priority genes out of 7470 genes across the 19 cancer types for advancing the field of cancer therapeutics [21].

These findings postulated that the effectiveness of approved oncology drugs in a given cancer type might be influenced by its ability to target or impair the functionality of specific

cellular targets as fitness genes. However, whether these cellular targets (of approved oncology drugs) are also fitness genes in other types of cancer remains unknown. Such an analysis will result in a broader utility of targeting specific cellular targets in additional cancer types and is being investigated here using the above CRISPR-Cas9-based cellular fitness dependency datasets [21,23]. In the present discovery and/or hypothesis-generating study, we bring out a global concept about the value of fitness genes across human cancer in the context of the utility of approved oncology drugs for cancer-types for which these drugs are approved or not approved.

2. Materials and Methods

2.1. Datasets

U.S. Food and Drug Administration approved oncology drugs during the period of 1952–September 2019 were collected from the FDA website (<https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm>, accessed on 13 September 2019). FDA approved oncology drugs searched on Bank databases (<https://www.drugbank.ca/>; version 5.1.4, accessed on 13 September 2019) [24–28] and the targets were identified. Fitness score for the gene targets were collected from the Cancer Dependency Map dataset (<https://score.depmap.sanger.ac.uk/gene>, accessed on 13 September 2019) [23].

2.2. U.S. Food and Drug Administration Approved Drugs

The Drug bank database mined for the targets of 235 oncology drugs included 185 small molecules, 5 enzymes and 45 biotechnology drugs. Among these targets, 230 are approved/re-approved after January 2000. Drug accession number, type of molecule, and weight of the molecule are collected and documented for each drug (Supplementary Table S1).

2.3. Drug-Target Data

Drug associated with 232 targets were extracted from the Drug bank database with one to one and one to many relationships. Drug targets included DNA, enzymes, protein complexes and genes. Among the target 109 genes of oncology drugs, 100 genes were found to be also present in the quality-control passed list of 7460 genes in the Cancer Dependency Map dataset [23].

2.4. Cell-Fitness Data

Complete set of 7470 fitness genes from CRISPR-Cas9-mediated knock-out study in 324 cancer cell lines representing 19 cancer-types were compared with the oncology drug target data and obtained an 100 genes targets present in common. We analyzed the fitness dependency of these genes using Cancer Dependency Map database. A set of 47 targets of FDA approved drugs were showing significant loss of fitness in corresponding cell lines were collected and categorized for cancer types based on the cell line model.

2.5. Fitness Score Data Analysis

Fitness scores for each target for cancer types were plotted using in-house R implementation. Multiple analysis for fitness scores to compare the targets for same cancer type, multiple cancer type for same cancer types etc. were performed and plotted to interpret results.

2.6. Genome Alterations and Gene Expression Analysis

Genome alterations and gene expression analysis for selected genes in the corresponding cancer datasets were performed using the cBioPortal.org [29,30] and Xena Browser [31] and other curated cancer dataset from Gene Expression Omnibus. The data sets were downloaded, normalized and converted to log expression values and thereafter represented the expression of the genes using the heatmap package of R bioconductor (<https://www.bioconductor.org>, version 3.12, accessed on 10 August 2020) and Genepat-

tern heatmap algorithms (<https://notebook.genepattern.org>, accessed on 8 June 2020). Alteration graph was directly exported from the cBioPortal web browser.

2.7. Survival Analysis

Survival plots for selected genes for corresponding datasets were performed using the SurvExpress tool [32] using default parameters.

2.8. Drug Target Cancer Relationship Diagram

Drug Target relationship for FDA approved oncology drugs for approved cancer types as well as significant fitness cancer types was identified and represented as Sankey chart diagram using Sankey MATIC tool (<http://sankeymatic.com>, accessed on 11 December 2020).

3. Results

3.1. Fitness Dependency of Cellular Targets of Oncology Drugs in Cancer Cell Lines

To define the global significance of cellular targets/effectors of oncology drugs in cancer cell growth, we examined the fitness dependency of known cellular targets and/or effectors of oncology drugs in cancer cell lines, that is, the requirement of a given gene for cell viability or cell growth. First, we examined the presence of 232 cellular targets known to be utilized by 235 FDA-approved oncology drugs (Supplementary Table S1) in the CRISPR-Cas9 fitness screen datasets [21] involving 324 validated cell lines [22,23]. We detected 100 out of 232 cellular targets in the database (Supplementary Table S2). Our analysis of the cancer-dependency screen identified 47 of these 100 cancer targets and/or effectors (of FDA-approved drugs) as fitness genes across 19 cancer types (Supplementary Figure S1A,B) and remaining 53 cellular molecules were identified without any loss of cellular fitness upon knocking out a specific molecules (Supplementary Tables S3 and S4).

We focused on the 47 cellular cancer target and/or effector genes (targeted by FDA approved drugs) in the subsequent studies. We observed that 15 of the 47 cellular targets of oncology drugs overlapped with recently identified 628 priority therapeutic targets [21] (Figure 1A, Supplementary Table S5). Both the 47 cellular targets of FDA-approved drugs and their subset of 15 cellular targets shared with priority therapeutic targets [21] were distributed across cancer types for which drugs targeting these cellular targets were either not approved (Figure 1B) or approved (Supplementary Figure S1C). Of the 15 cellular targets shared with the priority therapeutic targets, 10 molecules were targeted by small molecules, and 3 molecules were targeted by therapeutic antibodies (Supplementary Tables S6 and S7). These observations not only confirmed the recent findings of cellular target detection of approved cancer drugs as priority therapeutic targets [21] but also recognized 43 cellular targets and/or effectors with an excellent fitness effect in cancers for which drugs acting on these molecules are not approved (Supplementary Table S8). Moreover, 53 cellular targets and/or effectors of oncology drugs were without any effect on the cellular fitness effect upon their depletion (Figure 1A). Additionally, a small number of the 47 cellular molecules could be fitness genes in cancer-type context manner. For example, phosphoribosylglycinamide formyl transferase (GART) is an excellent fitness gene in ovarian cancer, for which pemetrexed targeting GART was approved; however, GART is not a fitness gene for lung and kidney cancers.

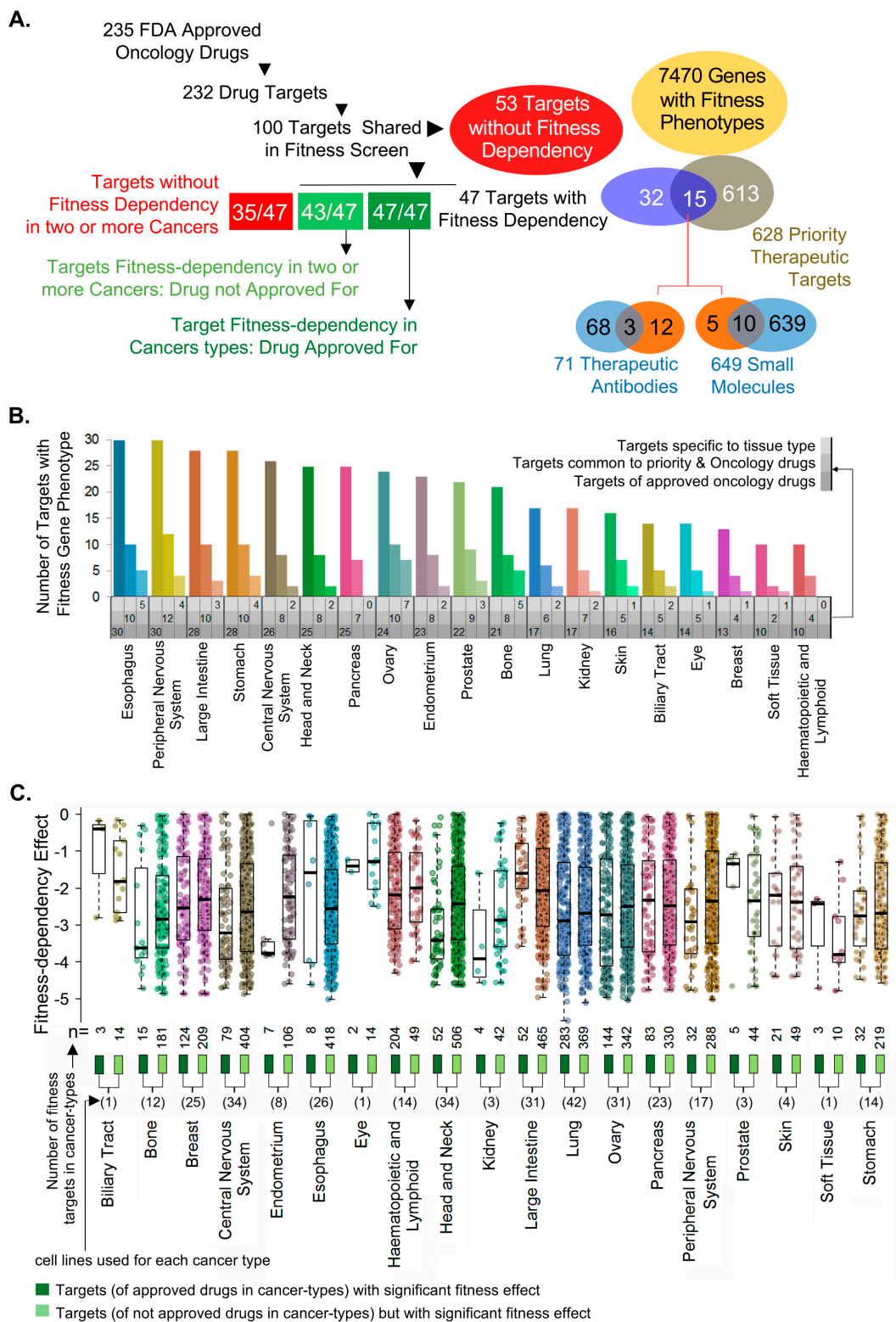


Figure 1. Oncology drug targets and/or effectors as good or poor cellular fitness genes. (A) Strategy to examine the fitness-dependency of cancer types for which oncology drugs targeting these targets are either approved or not approved. (B) Distribution of 43 cancer targets and/or effectors of FDA-approved drugs, a subset of 14 targets shared with 628 priority therapeutic targets and common targets between these two groups across cancer types, for which drugs targeting these cellular targets are not approved. (C) Distribution of significant fitness dependency of 47 molecules across 19 cancer types, for which drugs targeting these molecules are either approved (dark green boxes) or not approved (light green boxes). Here, n—collective number of target fitness values among cancer cell lines in a given cancer type; one dot per target per cell line.

To determine the requirement of the 47 known cellular targets and/or effectors (of cancer drugs) for the cellular fitness of cancer types, we examined the fitness dependency of these 47 genes in the CRISPR-Cas9-derived Cancer Dependency Map [23]. We observed that the individual depletion of these molecules in appropriate cancer types for which the drugs targeting these cellular molecules were approved resulted in a significant loss of cell fitness, thereby implying a role of these cellular targets in the growth of these cancer cell types (Figure 1C, dark green boxes; Supplementary Figure S1D). For example, the depletion of 13 targets (i.e., RRM1, TOP2A, TYMS, etc.) in breast cancer cells, 8 targets (i.e., RRM1, TOP1, MTOR, etc.) in glioblastoma cells, and 6 targets (i.e., TYMS, RRM1, TOP1, etc.) in pancreatic cancer cells resulted in a significant loss of cellular fitness, as depicted by the negative fitness effect. The depletion of 43 cellular molecules in cancer types for which drugs targeting these molecules were not approved also resulted in the loss of cell fitness (Figure 1C, light green boxes), suggesting that targeting or inactivating the functions of these 43 cellular molecules in these cancer types may also lead to growth inhibition. However, it's worth mentioning that mere elevation of these targets in certain cancers and their noted correlation with the disease outcome are not indicative of a direct causative effect [14]. In general, the number of dots corresponds to light green boxes, representative the effect of knocking out a single target gene in a given cancer cell line. The number of light green dots are substantially more than the number of dots corresponds to dark green. This suggests that the depletion and/or inactivating these 43 cellular molecules by appropriate targeting agents will have a fitness effect in these cancer cell lines.

3.2. Cellular Targets of Oncology Drugs Do Not Always Exhibit Fitness Dependency

In the next set of studies, we analyzed the effect of knocking out 53 cellular genes (previously known to be targeted by oncology drugs) across 19 cancer types and found that the deletion of these genes did not result in a significant loss of cellular fitness in multiple cell types (Figure 2A). Similar to these cellular targets, knocking out 35 of the 47 fitness genes was not accompanied by a loss of cell fitness in any one of the cancer type for which drugs targeting these molecules were approved (Figure 2B). For example, knocking out of the Fc Fragment of IgG receptor isoforms (FCGR1A, FCGR2B, and FCGR3a)– which are required for the manifestation of antibody-dependent cellular cytotoxicity and anti-angiogenic activity of bevacizumab [33], which targets the vascular endothelial growth factor receptor (VEGFR) in ovarian, intestinal, and kidney cancers, did not influence the cancer cell fitness (Figure 2C). Similarly, knocking out of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) (targets of palbociclib or ibrancein in breast cancer), ERBB2 (target of trastuzumab in breast cancer), Bruton's tyrosine kinase (BTK) (target of ibrutinib or imbruvica in hematopoietic cancer), CRBN (target of lenalidomide or revlimid in skin cancer), and CYP17A1 (target of abiraterone acetate or zytiga in prostate cancer) did not influence the fitness of cancer cell lines (Figure 2D). In contrast, we noticed that the depletion of such cellular genes was often accompanied by significantly improved fitness in cancer types (without the loss of cellular fitness), implying an enhanced cell growth of certain cancer-types if such genes are targeted in these cancer type. This raises some concern as targeting such molecules might potentially lead to a cell survival/proliferative response.

A number of recent reports demonstrated growth-promoting activities of oncology drugs in physiologically relevant whole animal models [34–38]. These observations raised two important possibilities for targeted cancer therapy. First, beneficial antitumor and therapy-associated toxic effects may result from off-target effects of certain oncology drugs if the intended drug target is not a fitness gene for the cell growth or cell viability in certain cancer types. Second, in the absence or loss of fitness dependency, attempts to inhibit such cellular target genes may lead to the increased proliferation of certain cancer cell types through indirect pathways. This possibility implies that if intended cellular drug targets are not affected by drugs, this could lead to undesirable effects in some cancer types.

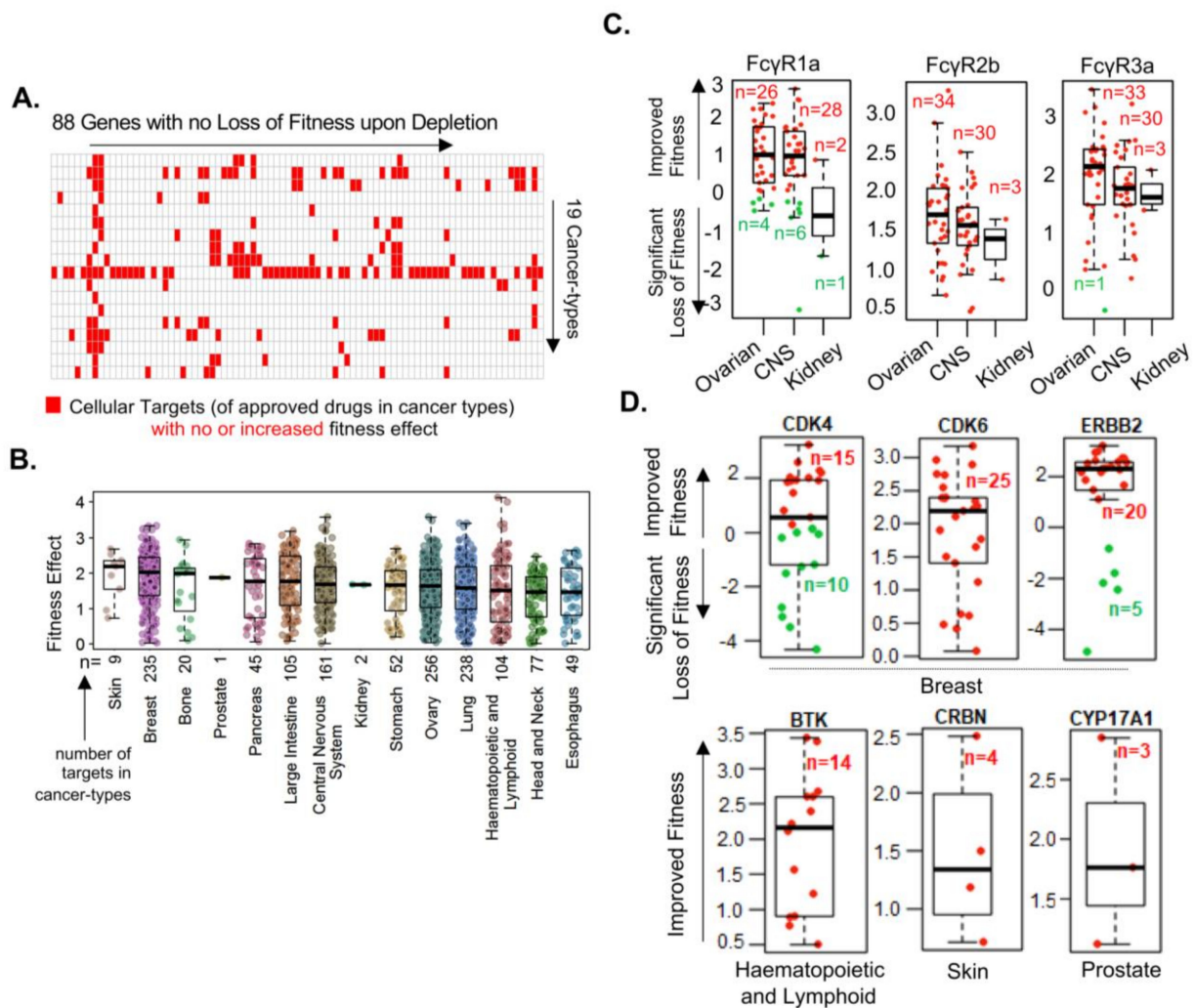


Figure 2. Revelation of cellular molecules with differential effects on cellular fitness. (A) Overall distribution of the 88 cancer targets with no loss of cellular fitness upon depletion across the 19 types of cancer cell lines. (B) Distribution of the positive fitness effect of depleting the 47 targets across cancer types, for which drugs utilizing these molecules are either approved or not approved. Here, n—collective number of target fitness values among cancer cell lines in a given cancer type; one dot per target per cell line. (C) Representative examples of the three fitness genes important in the action of bevacizumab in the three referred cancer types. (D) Selected examples of the above representative targets in cancer-types for which drugs targeting these molecules are approved using data from Drug bank and corresponding Fitness score for were derived from the Cancer Dependency Map.

3.3. Cellular Targets of Oncology Drugs Are Excellent Fitness Genes in New Cancer Types

To reveal a broader significance of the 47 cellular molecules which exhibited a significant fitness dependency in multiple cancer-types, we determined whether these molecules are required for the fitness or growth of cancer types for which drugs targeting these molecules were not approved. We found that the depletion of 43 of the 47 cellular molecules in multiple cancer types was associated with a substantial loss of cell fitness (Figure 1C and Figure 3A). Further, a direct comparison of the status of cellular targets and/or effectors which otherwise are known to be targeted by drugs that are approved or not approved for a given cancer, revealed that majority of cellular molecules with significant fitness dependency are present in cancer types for which drugs targeting these molecules are not approved for that cancer. Figure 3B illustrates the distribution of the fitness dependency scores of molecules which are targets and/or effectors of approved (dark green) or not approved (light green) drugs for breast cancer, pancreatic cancer, and glioblastoma. The

fitness dependency of the 43 targets of approved oncology drugs across Peripheral Nervous System, Large Intestine and Ovarian cancers is presented in Supplementary Figure S2.

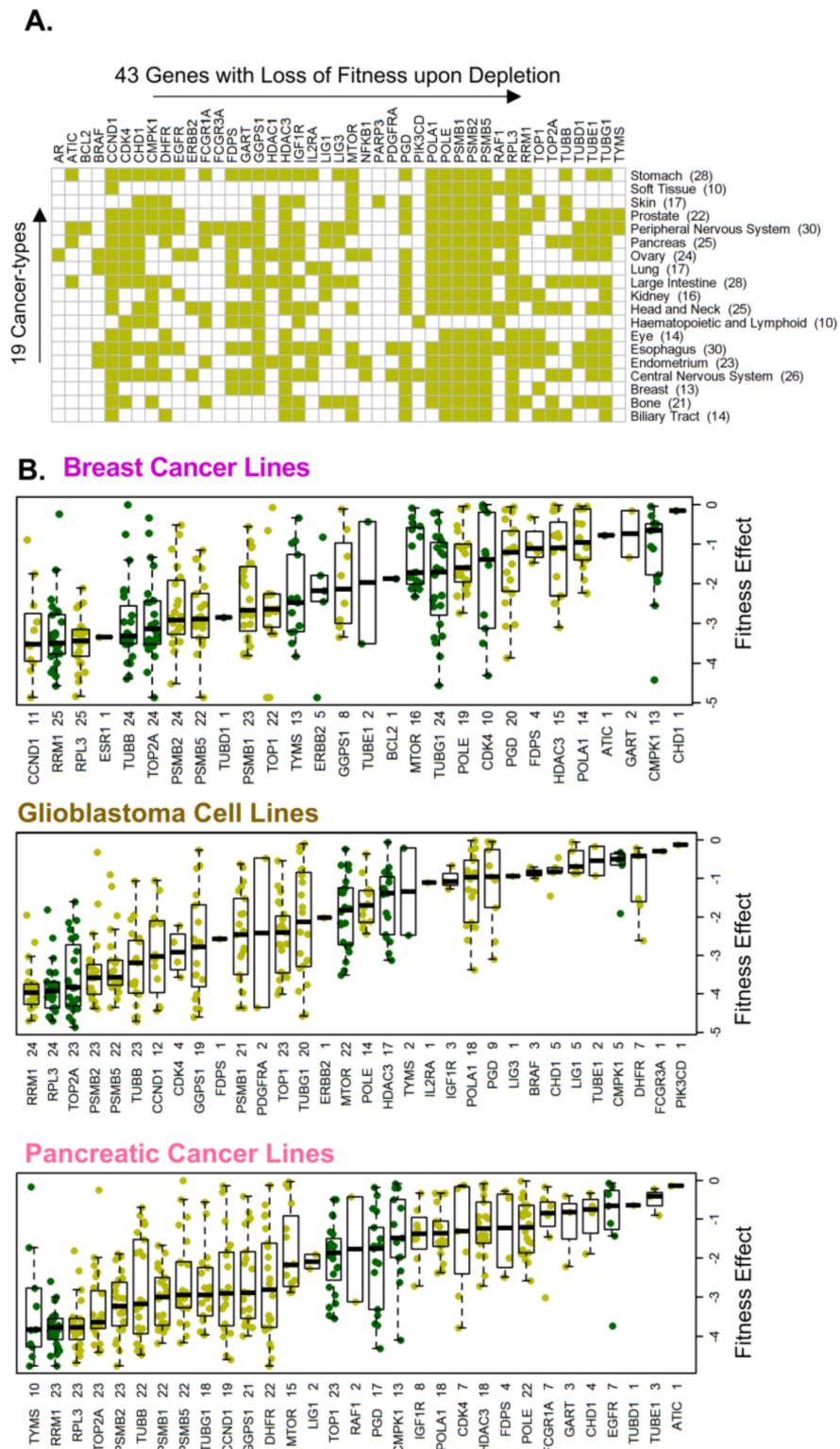


Figure 3. Distribution of fitness genes in a sub-set of cancer types. (A) Distribution of the 43 cancer cell fitness genes with a significant loss of cellular fitness upon depletion across the 19 cancer types. (B) Distribution of the loss of cellular fitness upon depletion of targets of either approved (dark green) or not approved (light green) oncology drugs in breast cancer, pancreatic cancer, or glioblastoma.

In general, cancer drug targets and/or effectors exhibited a widespread fitness dependency in cancer types for which drugs targeting these cellular targets are not approved (light green) compared with that in cancer types for which drugs targeting such molecules are approved (dark green) (Supplementary Figure S3). For example, the cellular fitness of breast, ovarian, and endometrial cancer cell lines was significantly compromised by the depletion of 13 (GGPS1, Farnesyl Diphosphate Synthase (FDPS), GART, and others), 24 (GGPS1, FCGR1A, TUBD1, and others), and 23 (GGPS1, FDPS, BRAF, and others) molecules, utilized by approved oncology drugs, respectively (Figure 3, Supplementary Figure S4A).

A multivariate analysis of tumors for overexpression versus underexpression of these fitness genes was associated with a highly significant reduction in the overall survival of respective cancer patients (Supplementary Figure S4B,C). Similarly, the fitness of esophageal, pancreatic, and stomach cancer cell lines was significantly compromised by the depletion of 30, 25, and 28 genes, respectively (Supplementary Figure S5A). A multi-variant analysis of overexpression versus underexpression of these fitness genes was also associated with a highly significant overall reduction in the survival of respective cancer patients (Supplementary Figure S5B,C). All 43 newly recognized fitness genes are otherwise known targets and/or effectors of FDA-approved drugs in referred cancer types for which drugs targeting these molecules are not approved (Supplementary Table S8). These results revealed the significance of cellular targets and/or effectors of approved oncology drugs in cellular fitness for cell viability in certain cancer types, raising the probability of repurposing certain cancer drugs for a set of new cancer types for which these drugs are not approved. However, depletion of such cellular targets and/or effectors in such cancer types revealed a significant fitness dependency.

3.4. Components of the Mevalonate Pathway as Fitness Genes in Breast Cancer

To study women's cancer, we evaluated the expression of 13 targets and/or effectors of cancer drugs, of which 10 are common among breast, ovarian, and endometrial cancers, with a significant fitness dependency in breast cancer (Supplementary Figures S2 and S4A). Among these cell fitness molecules, we observed a widespread mRNA overexpression and copy number amplification of Geranylgeranyl pyrophosphate synthase (GGPS1), Farnesyl diphosphate synthase (FDPS), and GART (also known as glycinamide ribonucleotide formyl transferase—GARFT) in breast tumors (Figure 4A and Supplementary Figure S6). The noted upregulation of GGPS1 and FDPS is an important observation in the context of breast cancer pathogenesis as these enzymes are components of the mevalonate pathway with role in cholesterol biosynthesis and bone metastasis of breast cancer, prostate cancer, and multiple myeloma [39–44]. The GART protein [45] is a mandatory trifunctional enzyme which plays an essential role in purine biosynthesis (Supplementary Figure S7A). The levels of GGPS1, FDPS, and GART were significantly elevated in breast tumors (Figure 4B) as compared to matching adjacent normal tissues [46], breast cancer cell lines (Figure 4C), breast cancer subtypes (Figure 4D), and triple negative breast cancer (TNBC) compared with the normal adjacent tissue or non-TNBC tumors (Figure 4E) [47]. The overexpression of GGPS1, FDPS, and GART mRNAs and their respective proteins was observed in breast tumors (Figure 5A) [48], along with the coexpression of GGPS1, FDPS, and GART proteins in several of the same breast tumors (presented as empty blocks).

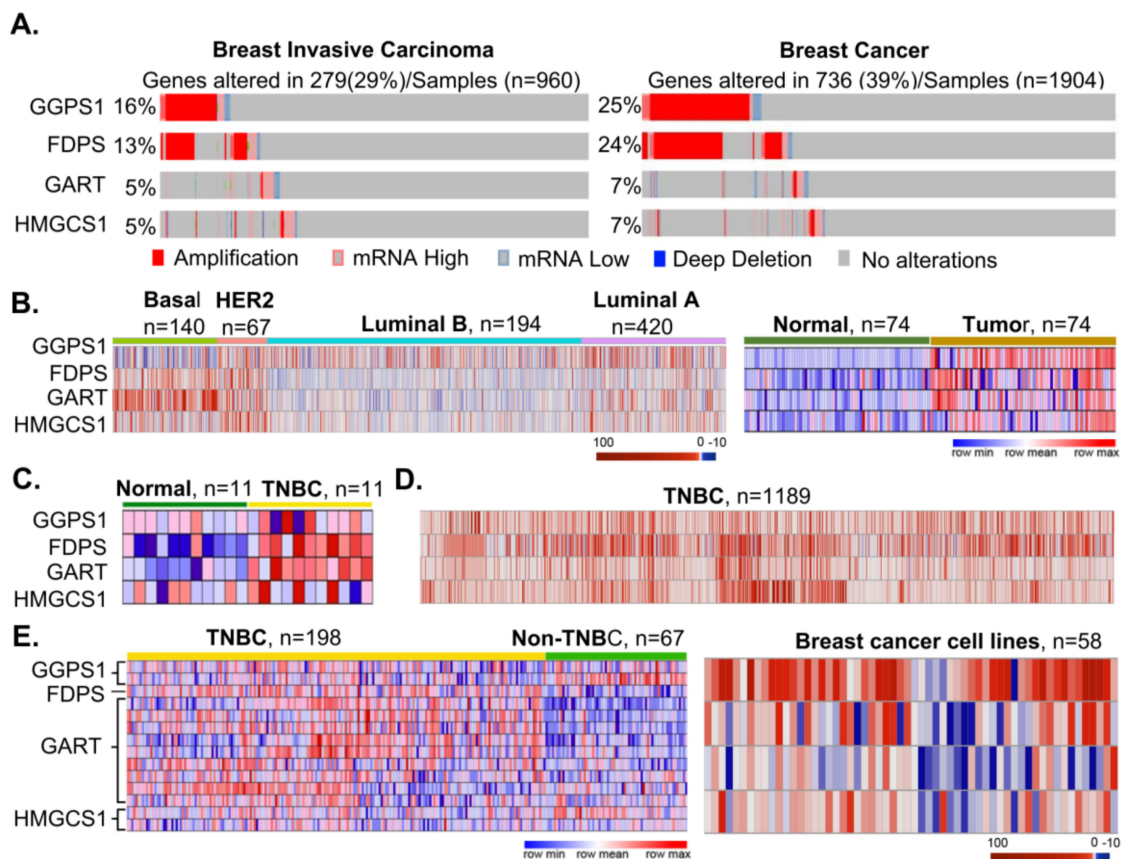


Figure 4. Expression of GGPS1, FDPS, HMGCS1 and GART in breast cancer. (A) Amplification and expression of indicated molecules in breast tumors in The Cancer Genome Atlas (TCGA) (left) and Metaberic data (right) [49] using Copy Number Variation (CNV) and gene alteration rate data from cBioPortal.org [29,30]. (B) Expression of GGPS1, FDPS, GART and 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1 (HMGCS1) mRNAs in breast cancer sub-types, in breast tumors and adjacent matched normal tissues using the data from cBioPortal (left panel) [29,30] and from Xena Browser (right panel) [31]. (C–E) Expression of indicated four mRNAs in TNBC and matched normal tissues from Xena Browser (right panel) [31], in TNBC samples [50], in TNBC and non-TNBC breast tumors, and breast cancer cell lines [51]; Right panel: Gene expression representation using heatmap in breast cancer cell lines—AU565, BT20, BT474, BT483, BT549, CAL120, CAL148, CAL51, CAL851, CAMA1, DU4475, EFM192A, EFM19, HCC1143, HCC1187, HCC1395, HCC1419, HCC1428, HCC1500, HCC1569, HCC1599, HCC1806, HCC1937, HCC1954, HCC202, HCC2157, HCC2218, HCC38, HCC70, HDQP1, HS274T, HS281T, HS343T, HS578T, HS606T, HS739T, HS742T, JIMT1, KPL1, MCF7, MDAMB134VI, MDAMB157, MDAMB175VII, MDAMB231, MDAMB361, MDAMB415, MDAMB436, MDAMB453, MDAMB468, SKBR3, T47D, UACC812, UACC893, YMB1, ZR751, ZR7530, EVSAT and HMC18 cells using data from cBioPortal.org [29,30].

GGPS1 and FDPS are targets of nitrogen-containing bisphosphonates such as zoledronic acid derivatives that are widely used to prevent bone-related events related to breast cancer relapse. These drugs reduce mortality in postmenopausal women through the inhibition of bone metastasis by suppressing osteoclast-mediated bone resorption [39–42]. This is achieved by inhibiting osteoclastic activity and decreasing the bone turnover as supported by reduction in the levels of bone resorption markers N-telopeptide and C-telopeptide [39–44]. Bisphosphonates are generally considered supportive therapy and not anti-cancer therapy for solid tumors due to a modest modifying effect on the overall survival of patients with solid tumors in clinical trials undertaken by two of the authors of this study [42–44]. Bisphosphonates also increase the overall survival in multiple myeloma [52]. However, the nature and context of cellular targets of bisphosphonates in breast cancer (GGPS1 and FDPS) are expected to be different from its targets in bone.

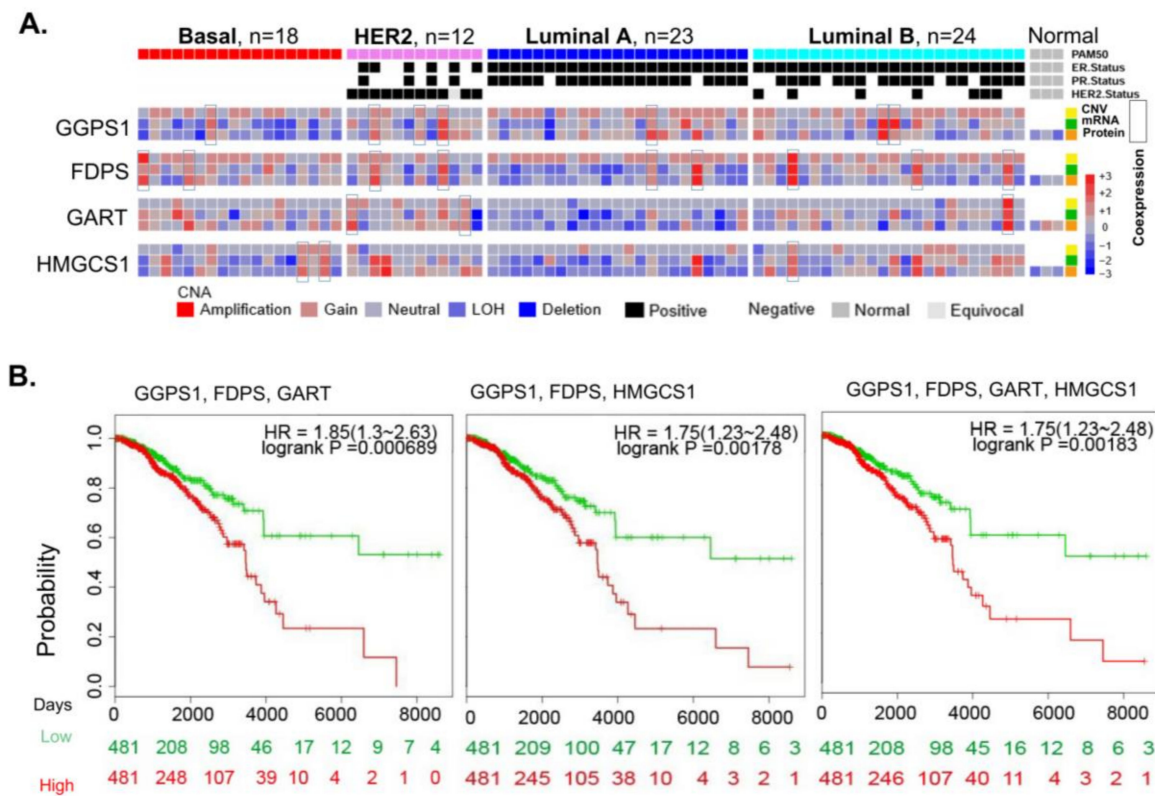


Figure 5. Coexpression and significance of GGPS1, FDPS, GART and HMGCS1 in breast cancer. (A) Proteogenomics expression status of the four indicated molecules in breast tumors. Yellow: CNV, Green: a RNaseq, and Orange: Protein [48]. (B) SurvExpress [32] survival analysis of GGPS1, FDPS, and GART and that of GGPS1, FDPS, GART, and HMGCS1 in patients with breast tumors.

A recently completed clinical trial, named AZURE trial, postulated certain beneficial antitumor activity of bisphosphonates against breast cancer in the adjuvant setting in a subset of postmenopausal women who were negative for MAF transcription factor [53,54]. Previously studies have shown that MAF regulates the expression of genes important in breast-to-bone metastasis [55–57]. We next analyzed the relationship between the levels of MAF and bisphosphonate’s targets in breast tumors. We noticed an inverse relationship between levels of MAF upregulation and those of GGPS1 or FDPS, and an almost exclusive expression of MAF and GGPS1 or MAF and FDPS in breast tumors (Supplementary Figure S8A). We also found that MAF is not a fitness gene because its depletion in cancer cell lines had no effect on cellular viability (Supplementary Figure S8B). It remains an open question whether the responders to bisphosphonates in the AZURE trial were positive for GGPS1 and/or FDPS—both of which have been implicated in oncogenesis [58–60], and thus, MAF-negative breast tumors might have responded well to bisphosphonate therapy due to the presence of its targets—a question for validation in a prospective clinical study.

Zoledronic acid acts by inhibiting these enzymes due to its analogous nature with naturally occurring pyrophosphates and by suppressing the geranylgeranylation and farnesylation of small GTPases (Supplementary Figure S7A). Antifolates such as pemetrexed, which are approved for ovarian and kidney cancers, target GART, dihydrofolate reductase, and thymidylate synthase [45]. The overexpression of GGPS1, FDPS, and GART in breast cancer was also associated with a highly significant overall reduction in the survival of patients with breast cancer compared with that of patients without overexpression of these cellular molecules (Figure 5B). The significance of the overexpression of GGPS1, FDPS, and GART in the pathophysiology of breast cancer is also evident by the fitness dependency of breast cancer cells on these genes (Figure 5B). As most of relapses occur in the first 5 years,

it would be interesting to understand whether these fitness genes are responsible for cancer progression, or recurrence of cancer in future studies.

3.5. Components of the Mevalonate Pathway as Fitness Genes in Hard-to-Treat Cancer Types

GGPS1, FDPS, and GART are upregulated in multiple cancers in addition to breast cancer (also TNBC) (Figures 4 and 5, Supplementary Figure S9), including hard-to-treat cancers such as esophageal, pancreatic, lung, and oral cancers and glioblastoma (Figure 2, Supplementary Figures S3 and S9–S11). The potential significance of the noticed overexpression of GGPS1, FDPS, and GART in the pathophysiology of cancer types, other than breast cancer, is evident by the fitness dependency of multiple hard-to-treat cancer cell types such as esophageal, central nervous system (CNS), head and neck, and ovarian cancers, on the presence of GGPS1, FDPS, and GART (Figure 6A,B).

Additionally, the overexpression of GGPS1, FDPS, GART, and 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) was also associated with a highly significant overall reduction in the survival duration of patients with esophageal and pancreatic cancers. Similarly, the overexpression of GGPS1, GART, and HMGCS1 reduced the overall survival duration in patients with glioblastoma, ovarian cancer, and endometrial cancer but not in those with prostate cancer (Supplementary Figure S10). We found that the levels of GGPS1, FDPS, and GART were not upregulated in prostate cancer (Supplementary Figure S10). GGPS1 did not exhibit any fitness dependency in prostate cancer cells, whereas FDPS and GART had no fitness values (Supplementary Figure S10). Zoledronic acid is also used for prostate cancer bone metastases, suggesting that some degree of cell-type specificity of fitness dependency of the same set of genes may exist between breast and prostate cancer cells for presently obscure reasons.

FDPS and GGPS1 are downstream components of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (Supplementary Figure S7A), a rate-limiting enzyme and a target of statins [61]. The statin treatment of cancer cells leads to a compensatory upregulation of HMGCS1, which is widely upregulated in breast [58,60] and other cancer-types (Figures 4 and 5; Supplementary Figures S6 and S9). Although HMGCS1 is not a target of any FDA-approved oncology drug, the knock-out of HMGCS1 in fitness screens was accompanied by a significant fitness dependency of breast cancer and other cancer cell types (Figure 4A,B). We also observed that the overexpression of HMGCS1, GGPS1, FDPS, and GART in breast (Figure 4), ovarian, endometrial, pancreatic, and CNS cancers (Supplementary Figure S10) correlated well with a reduction in the overall survival of patients when compared with that of patients without overexpression of these genes.

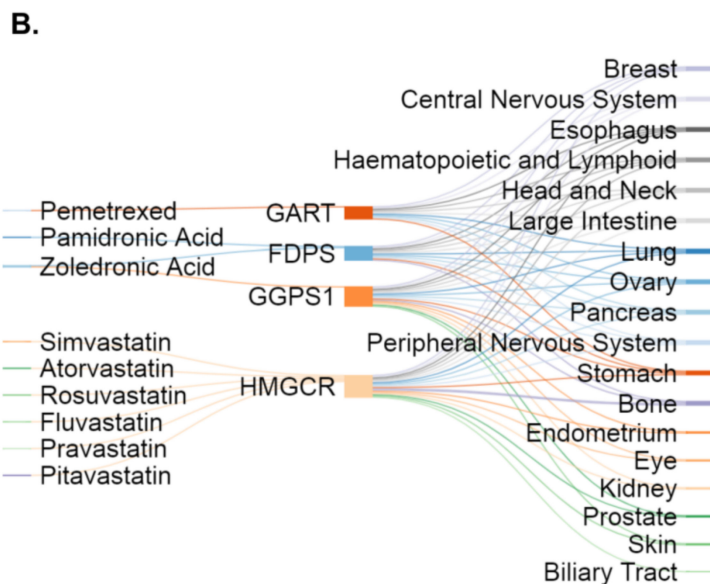
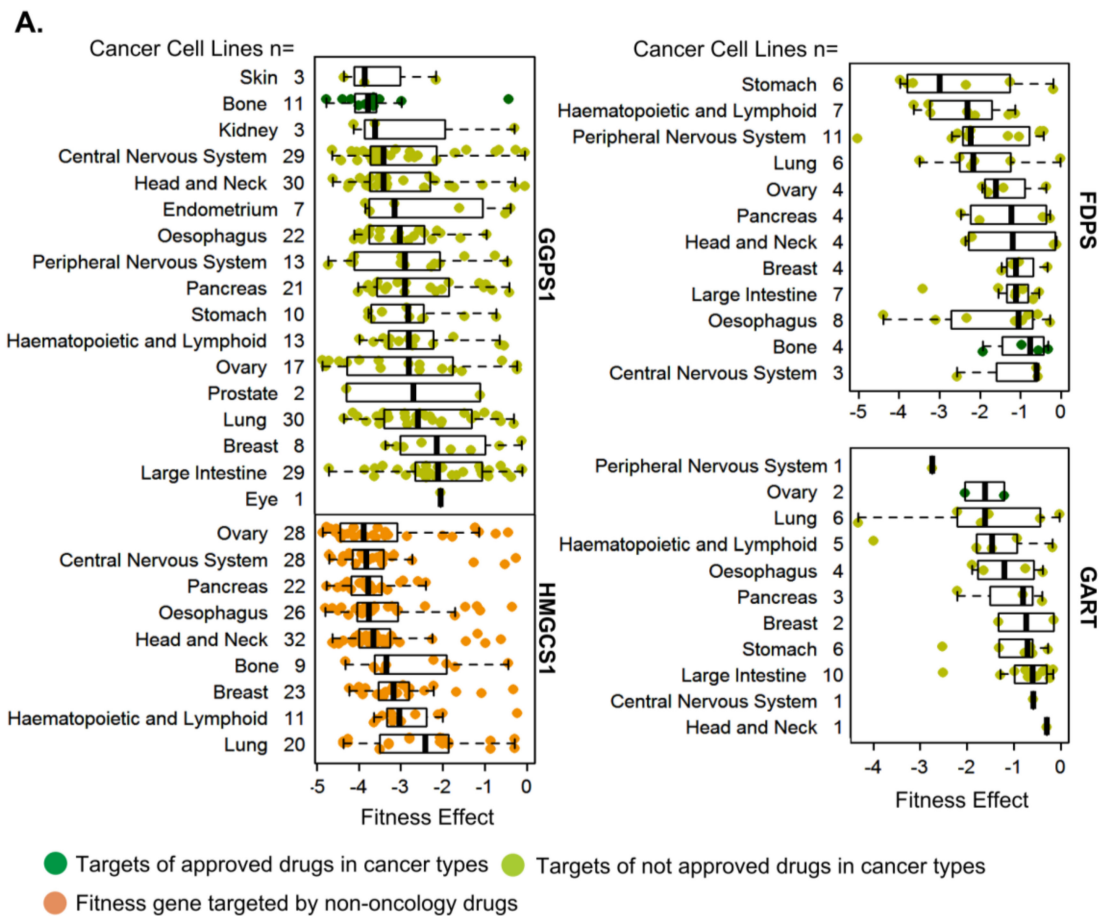


Figure 6. Fitness dependency of the four enzymes of the mevalonate/cholesterol pathway in cancer-types. **(A)** Status of cellular fitness of cancer types upon knocking out GGPS1, FDPS, HMGCS1, or GART in cancer types for which drugs utilizing these molecules are approved (dark green dots) or not approved (light green) or are non-oncology drugs (orange). **(B)** Relationship between the four cellular targets or effectors and fitness dependency of cancer types, for which indicated drugs targeting these molecules are not approved.

4. Discussion

The present study encouraged the utilization of post-genomic data for the benefit of patients with cancer by repurposing approved cancer drugs by integrating fitness dependency of the intended target and/effector of a given oncology drug. Summary of the work presented here in Figure 7 illustrates a substantial increase in the number of fitness gene targets in cancer types for which oncology drugs targeting these targets are not approved compared with cancer types for which these drugs are approved—as evident by the number of lines connecting the target and cancer types. These results support the notion of utilizing the genomic data for the benefit of patients with cancer by repurposing approved cancer drugs by integrating fitness dependency of the intended target, its cellular overexpression, and role in the overall survival of patients with high versus low expression of fitness genes in multi-variant analyses.

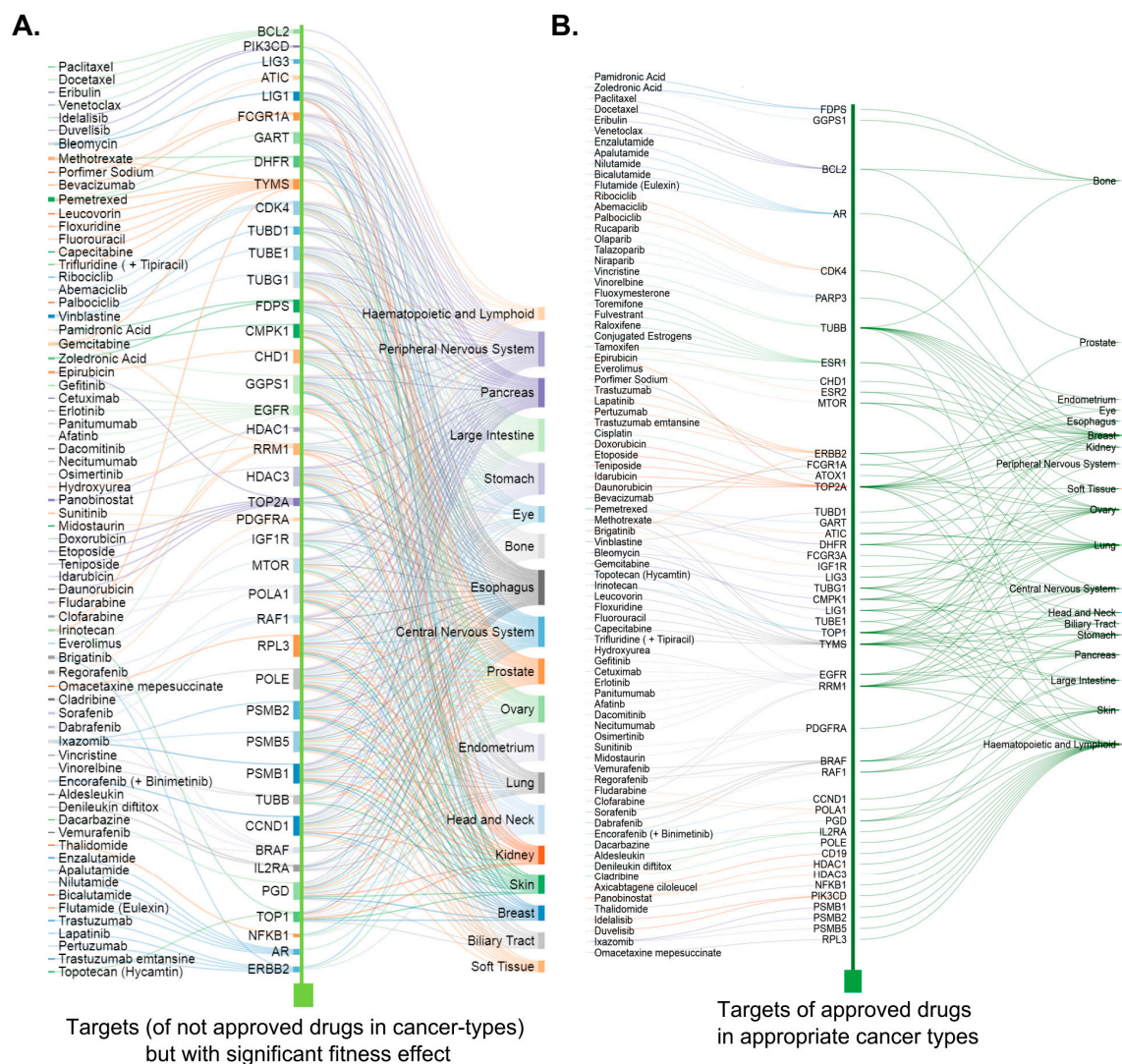


Figure 7. Relationship between the cellular targets and/or effectors of oncology drugs and cancer-types. Overall summary of the relationship noticed in the present study between the cancer types and cellular targets or effectors with significant fitness-dependency for which indicated drugs targeting these molecules are not approved (A) or approved (B).

To exemplify the usefulness of the findings presented here, we focused on breast cancer and demonstrate that targets of bisphosphonates such as FDPS and GGPS1 as well as statin such as HMGCS1 are excellent fitness genes in breast cancer and many hard-to-treat cancers. Because a large body of prior data suggests that use of statins may be

associated with a reduced incidence of breast as well as esophageal cancer etc. [62–66] and the fact that all four enzymes, i.e., GGPS1, FDPS, HMGCS1 and HMGCR, belong to the mevalonate pathway, these observations might offer some degree of scientific reasoning for potentially combining bisphosphonates with statins (along with strategies to target HMGCS1) for cancer-types for which these drugs are not approved (Figure 6B). However, completed clinical trials have not prescreened the patients for the status or activity of cellular targets of bisphosphonates (i.e., FDPS and GGPS1) and statins (HMGCR and HMGCS1)—a premise for targeted therapy. The combination of bisphosphonates and statins (probably with or without pemetrexed) may yield a superior therapy response in a subset of cancers such as TNBC and ovarian, pancreatic, and CNS cancer if such patients are stratified on the basis of the expression of FDPS, GGPS1, HMGCR, and GART in future clinical trials (Figure 6B). Zoledronic acid exhibits synergistic growth inhibitory activity with other anticancer agents in cellular models [67]. A recently completed breast cancer clinical trial aimed at repurposing zoledronic acid in a neoadjuvant setting suggests that zoledronic acid promotes the anticancer activity of chemotherapy and antiHER2 therapy [68]. Bisphosphonates or statins have been proven to be safe when used over an extended period of time. The expression of GGPS1, FDPS, GART, and HMGCS1 is very low or albeit in the normal cell types and immune cell types (Supplementary Figure S11), and in human blood cells (Supplementary Figure S12)

The findings presented here can impact the field of cancer drug repurposing and provide new hypotheses to be tested in future studies using appropriate preclinical model systems and subsequently, a novel cancer-specific clinical trial. In addition to repurposing bisphosphonates and statins, with or without pemetrexed, for breast cancer (and perhaps, other cancer-types), the present analysis provided a rationale for repurposing a range of approved oncology drugs in cancer-types for which these drugs are not approved, but their cellular targets are excellent fitness genes in these cancer-types. The potential relationship between the levels of cancer therapeutic targets in serum, plasma, and tumors might also be of interest because certain targets and/or effectors of oncology drugs were also detected in extracellular fluids as secretory proteins (FDPS, GART, and HMGCS1) [69], in addition to being fitness genes and overexpression in human tumors. Such secretory fitness gene products could be potentially developed as surrogate biomarkers for the assessment of disease status and therapeutic responsiveness.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4409/10/2/433/s1>, Figure S1: Fitness-dependency of cellular targets of approved oncology drugs in cancer cell lines, Figure S2: Cellular targets of approved oncology drugs as excellent fitness genes in cancer-types for which drugs targeting these are not approved, Figure S3: Status of fitness-dependency of 47 oncology drug targets in cancer-dependency map, Figure S4: Widespread overexpression and significance of cellular fitness targets in women’s cancer, Figure S5: Widespread overexpression and significance of cellular fitness targets in digestive cancers, Figure S6: CNV amplification of GGPS1, FDPS, GART, and HMGCS1 in representative human cancers, Figure S7: Pathways and molecules of interest in the present study, Figure S8: An inverse relationship between the levels of MAF versus GGPS1 or FDPS, Figure S9: Expression of GGPS1, FDPS, GART and HMGCS1 in different TNBC studies, Figure S10: Expression of GGPS1, FDPS, GART and HMGCS1 in hard-to-treat cancers, Figure S11: Status of GGPS1, FDPS, GART and HMGCS1 in physiologic settings, Figure S12: Expression of GGPS1, GART, FDPS and HMGCS1 mRNAs in blood cells. Table S1: The list of 235 FDA approved drug targets for cancer treatment, Table S2: Drug targets and associated drug from Drugbank, Table S3: Oncology drug targets present in Cancer Dependency Map dataset, Table S4: Fitness genes across 19 cancer-types in the cancer-dependency screen, Table S5: List of 15 fitness genes common with 628 priority genes across 19 cancer-types, Table S6: List of 10 fitness genes common with therapeutic small molecules, Table S7: List of 3 fitness genes common with molecules targeted by therapeutic antibodies, and Table S8: Cellular targets with excellent fitness effect in cancer-types for which drugs targeting these targets are not approved [70–79].

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B.G., R.K., M.R.P., K.L., S.M.A. and O.S., A.L., and P.R.; Investigation, B.G., A.M.P. and R.A.; Resources, R.K. and M.R.P.; Data Curation, B.G., P.M.P., A.M.P. and R.A.; Writing—Original Draft Preparation, B.G. and R.K.; Writing—Review & Editing, R.K., A.L., P.R., G.N.H. and M.R.P.; Visualization, B.G. and R.K.; Supervision, R.K.; Project Administration, R.K.; Funding Acquisition, M.R.P.; Lead Study Contact, R.K. All authors have read and agreed to the published version of the manuscript.

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