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**Computational pharmacogenomic screen identifies drugs that phenocopy dipyridamole and
potentiate anti-breast cancer activity of statins**

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22 Abstract

23 Statins are a family of FDA-approved cholesterol-lowering drugs that inhibit the rate-limiting enzyme of
24 the metabolic mevalonate pathway, which have been shown to have anti-cancer activity. As therapeutic
25 efficacy increases when drugs are used in combination, we sought to identify agents, like dipyridamole,
26 that potentiate statin-induced tumor cell death. As an antiplatelet agent dipyridamole will not be suitable
27 for all cancer patients. Thus, we developed an integrative pharmacogenomics pipeline to identify agents
28 that were similar to dipyridamole at the level of drug structure, *in vitro* sensitivity and molecular
29 perturbation. To enrich for compounds expected to target the mevalonate pathway, we took a
30 pathway-centric approach towards computational selection, which we called mevalonate drug network
31 fusion (MVA-DNF). We validated two of the top ranked compounds, nelfinavir and honokiol and
32 demonstrated that, like dipyridamole, they synergize with fluvastatin to potentiate tumour cell death by
33 blocking the restorative feedback loop. This is achieved by inhibiting activation of the key transcription
34 factor that induces mevalonate pathway gene transcription, sterol regulatory element-binding protein 2
35 (SREBP2). Mechanistically, the synergistic response of fluvastatin-nelfinavir and fluvastatin-honokiol was
36 associated with similar transcriptomic and proteomic pathways, indicating a similar mechanism of action
37 between nelfinavir and honokiol when combined with fluvastatin. Further analysis identified the canonical
38 epithelial-mesenchymal transition (EMT) gene, E-cadherin as a biomarker of these synergistic responses
39 across a large panel of breast cancer cell lines. Thus, our computational pharmacogenomic approach can
40 identify novel compounds that phenocopy a compound of interest in a pathway-specific manner.

41 **Key words:** Drug combinations; cancer therapy; mevalonate pathway; drug similarity; drug perturbations;
42 pharmacogenomics; breast cancer

43 Significance Statement:

44 We provide a rapid and cost-effective strategy to expand a class of drugs with a similar phenotype. Our
45 parent compound, dipyridamole, potentiated statin-induced tumour cell death by blocking the
46 statin-triggered restorative feedback response that dampens statins pro-apoptotic activity. To identify
47 compounds with this activity we performed a pharmacogenomic analysis to distinguish agents similar to
48 dipyridamole in terms of structure, cell sensitivity and molecular perturbations. As dipyridamole has many
49 reported activities, we focused our molecular perturbation analysis on the pathway inhibited by statins,
50 the metabolic mevalonate pathway. Our strategy was successful as we validated nelfinavir and honokiol
51 as dipyridamole-like compounds at both the phenotypic and molecular levels. Our developed approach
52 sets the framework for future pathway-centric identification of drug combinations.

53 Introduction

54 Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer (BC) that has a poorer
55 prognosis amongst the major breast cancer subtypes¹. This poor prognosis stems from our limited
56 understanding of the underlying biology, the lack of targeted therapeutics, and the associated risk of
57 distant recurrence occurring predominantly in the first two years after diagnosis². Cytotoxic anthracycline
58 and taxane-based chemotherapy regimens remain the primary option for treating TNBC, with other
59 classes of investigational agents in various stages of development. Therefore, novel and effective
60 therapeutics are urgently needed to combat this difficult-to-treat cancer.

61 Altered cellular metabolism is a hallmark of cancer^{3,4} and targeting key metabolic pathways can
62 provide new anti-cancer therapeutic strategies. Aberrant activation of the metabolic mevalonate (MVA)
63 pathway is a hallmark of many cancers, including TNBC, as the end-products include cholesterol and
64 other non-sterol isoprenoids essential for cellular proliferation and survival⁵⁻⁷. The statin family of
65 FDA-approved cholesterol-lowering drugs are potent inhibitors of the rate-limiting enzyme of the MVA
66 pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)⁶. Epidemiological evidence shows that
67 statin-use as a cholesterol control agent is associated with reduced cancer incidence⁸ and recurrence⁹⁻¹³.
68 Specifically, in BC, a 30-60% reduction in recurrence is evident amongst statin users, and decreased risk
69 is associated with increased statin duration^{9,12,14,15}. We and others have shown preclinically that Estrogen
70 Receptor (ER)-negative BC cell lines, including TNBC, are preferentially sensitive to statin-induced
71 apoptosis^{16,17}. Moreover, three preoperative clinical trials investigating lipophilic statins (fluvastatin,
72 atorvastatin) in human BC, showed statin use was associated with reduced tumour cell proliferation and
73 increased apoptosis of high-grade BCs^{18,19}. Thus, evidence suggests that statins have potential utility in
74 the treatment of BC, including TNBC.

75 Drug combinations that overcome resistance mechanisms and maximize efficacy have potential
76 advantages as cancer therapy. Blocking the MVA pathway with statins triggers a restorative feedback
77 response that significantly dampens the pro-apoptotic activity of statins^{20,21}. Briefly, statin-induced

78 depletion of intracellular sterols, triggers the inactive cytoplasmic, precursor form of the transcription
79 factor sterol regulatory element-binding protein 2 (SREBP2) to be processed to the active mature nuclear
80 form, which induces transcription of MVA genes, including *HMGCR* and the upstream synthase
81 (*HMGCS1*)²². We have shown that inhibiting SREBP2 using RNAi, or blocking SREBP2 processing using
82 the drug dipyridamole, significantly potentiates the ability of statins to trigger tumor cell death^{21,23,24}.

83 Dipyridamole is an FDA-approved antiplatelet agent commonly used for secondary stroke
84 prevention, and since statin-dipyridamole has been co-prescribed for other indications, it may be safely
85 used in the treatment of cancer²⁵. However, the exact mechanism of dipyridamole action remains unclear
86 as it has been reported to regulate several biological processes. Moreover, the antiplatelet activity of
87 dipyridamole may be a contraindication for some cancer patients. Thus, to expand this dipyridamole-like
88 class of compounds that can potentiate the pro-apoptotic activity of statins, we employed a
89 pathway-centric approach to develop a computational pharmacogenomics pipeline to distinguish
90 compounds that are predicted to behave similarly to dipyridamole in the regulation of MVA pathway
91 genes. Using this strategy, we identified several potential dipyridamole-like compounds including
92 nelfinavir, an FDA-approved antiretroviral drug and honokiol, a compound isolated from *Magnolia spp.*,
93 which synergise with statins to drive tumour cell death by blocking the restorative feedback response.
94 Correlation analysis of the statin-compound combination synergy score, with basal mRNA expression
95 across a large panel of BC cell lines, identified *CDH1* expression as a predictive biomarker of response to
96 these combination therapies. Taken together, we provide a new strategy to identify compounds that
97 behave functionally similar to dipyridamole in an MVA pathway-specific manner and suggest that this
98 approach will have broad utility for compound discovery across a wide variety of drug/pathway
99 interactions.

100 Results

101 Computational pharmacogenomic pipeline identifies dipyridamole-like compounds

102 We developed a computational pipeline that harnesses high-throughput pharmacogenomics analysis to
103 identify dipyridamole-like compounds that synergise with statins by blocking MVA pathway gene

expression to inhibit cancer cell viability (**Figure 1A and 1B**). The LINCS-L1000 (L1000)²⁶ and NCI-60²⁷ datasets were chosen for these studies as they contain cellular drug-response data at the molecular and proliferative levels across a panel of cell lines, respectively. From these datasets we extracted drug structure, drug-induced gene perturbation data (gene expression changes after drug treatment) and drug-cell line sensitivity profiles for the 238 compounds common to both datasets. Treating each level of data as a separate layer, we restricted the drug-gene perturbation layer from the L1000 dataset to only include the six MVA pathway genes present in the L1000 landmark gene set, to enrich for compounds that phenocopy the MVA pathway-specific activity of dipyridamole (**Supplemental Figure 1A and Figure 1B**). With dipyridamole as the reference input, we generated an MVA pathway-specific Drug Network Fusion (MVA-DNF) through the integration of three distinct data layers: drug structure, MVA-specific drug perturbation signatures, and drug-cell line sensitivity profiles. For each of the data layers incorporated into MVA-DNF, a 238x238 drug affinity matrix was generated, indicating drug similarity for a selected drug against all other drugs (further described in methods). Briefly, we first computed similarity between pairs of drug structures using the Tanimoto index, prior to generating the structure affinity matrix. We computed the similarity for every pair of drug sensitivity profiles using the pearson correlation coefficient, prior to generating an affinity matrix for the drug sensitivity layer. To create an affinity matrix for the MVA-specific perturbation layer, we first calculated the pearson correlation coefficient on the drug perturbation signatures that were filtered to include only MVA genes (**Figure 1B**). By integrating the three affinity matrices using similarity network fusion, and filtering hits using permutation testing, we subsequently identified 23 potential dipyridamole-like compounds that scored as significant (permutation test p-value <0.05) (**Figure 1B, Supplementary Table 1**, and further explained in methods). Represented as a network, these hits display strong connectivity to dipyridamole as well as to each other.

We assessed the contribution of the different data layers (drug structure, drug-gene perturbation, and drug-cell line sensitivity) within the MVA-DNF for each of these 23 compounds (**Figure 1C**). Drug perturbation played a significant role in the selection of novel dipyridamole-like compounds compared to drug sensitivity and drug structure. This reflects upon the specificity of the MVA-DNF towards the MVA pathway, in comparison to a 'global' drug taxonomy that is not MVA pathway-centric. Further assessment of the six MVA-pathway gene expression changes within the drug perturbation signatures highlights

comparable expression profiles between dipyridamole and the novel dipyridamole-like compounds
(**Supplementary Figure 1B**).

To prioritize and further interrogate the identified dipyridamole-like hits we annotated the 23 compounds by reported mechanism of action and potential clinical utility. Two compounds were excluded from further analysis as they were not clinically useful: chromomycin A3, a reported toxin²⁸, and cadmium chloride, an established carcinogen²⁹. The remaining 21 compounds segregated into ten distinct categories, demonstrating that dipyridamole-like hits identified through our pharmacogenomics pipeline spanned a diverse chemical and biological space (**Supplemental Figure 1C, Supplemental Table 1**). We sought to validate the five hits that scored as most similar to dipyridamole, which belong to four different categories: RAF/MEK inhibitor (selumetinib); antiretroviral (nelfinavir); anthracycline (doxorubicin, mitoxantrone); and natural product (honokiol). The reliability of our approach is evidenced by previous work by our lab and others that the anthracycline doxorubicin potentiates lovastatin in ovarian cancer cells³⁰ and RAF/MEK inhibitors such as PD98059 and more recently selumetinib (AZD6244) have been reported to synergise with statins to potentiate cancer cell death^{31,32}. The molecular targeted compound (selumetinib) along with the novel three compounds were advanced for further evaluation (nelfinavir, mitoxantrone and honokiol) (**Supplemental Table 1**).

Dipyridamole-like compounds induce apoptosis in combination with fluvastatin and block the sterol-regulated feedback loop of the MVA pathway

To investigate whether the dipyridamole-like compounds could potentiate fluvastatin-induced cell death similar to that of dipyridamole, we first investigated sensitivity to increasing statin exposure in combination with a sub-lethal concentration of the novel dipyridamole-like compounds in two breast cancer cell line models with differential sensitivity to fluvastatin as a single agent (**Supplemental Figure 2**)¹⁶. As seen with dipyridamole, we observed similar potentiation of fluvastatin (lower IC₅₀) when combined with a sub-lethal concentration of selumetinib, nelfinavir, or honokiol, but not mitoxantrone (**Supplemental Figure 3 and Supplemental Figure 4**). Therefore, mitoxantrone was no longer pursued as a dipyridamole-like compound. To determine the nature of the anti-proliferative activity of the statin-compound combinations, we evaluated cell death by fixed propidium iodide staining and PARP cleavage with selumetinib, nelfinavir, or honokiol. Our data indicate that all three compounds, at

160 concentrations that have minimal effects as single agents, phenocopy dipyridamole and potentiate
161 statin-induced cell death (**Figure 2**).

162 Mechanistically, statins induce a feedback response mediated by SREBP2 that has been shown
163 to dampen cancer cell sensitivity to statin exposure. Moreover, blocking the SREBP2-mediated feedback
164 response with dipyridamole enhances statin-induced cancer cell death^{21,23}. We have shown that
165 dipyridamole blocks the regulatory cleavage and therefore activation of SREBP2, decreasing mRNA
166 expression of SREBP2-target genes of the MVA pathway. As expected, statin treatment induced the
167 expression of SREBP2-target genes, *INSIG1*, *HMGCR* and *HMGCS1* after 16 hr of treatment, which was
168 blocked by the co-treatment with dipyridamole (**Figure 3A, Supplemental Figure 5A**). Similarly, nelfinavir
169 and honokiol both phenocopy dipyridamole and block the statin-induced expression of MVA pathway
170 genes (**Figure 3A, Supplemental Figure 5A**). By contrast, co-treatment with selumetinib did not block
171 the fluvastatin-induced feedback response. Housekeeping gene RPL13A was used as a reference gene
172 for normalizing mRNA between samples and was not altered in the presence of the compounds
173 (**Supplemental Figure 5B**).

174 Because SREBP2 is synthesized as an inactive full-length precursor that is activated to the
175 mature nuclear form upon proteolytic cleavage, we used western blot analysis to assess the protein levels
176 of both full-length and mature SREBP2. Nelfinavir and honokiol, but not selumetinib, blocked
177 fluvastatin-induced SREBP2 processing and cleavage similar to that of dipyridamole (**Figure 3B-C**). This
178 suggests that while selumetinib is a strong potentiator of statin-induced cell death, it does not mimic the
179 action of dipyridamole by blocking the restorative feedback response (**Figure 3, Supplemental Figure 5**).

180 **Novel statin-compound combinations phenocopy synergistic activity of fluvastatin-dipyridamole** 181 **in a breast cancer cell line screen**

182 To investigate whether the potentiation of fluvastatin by nelfinavir and honokiol has broad applicability and
183 examine the determinants of synergy, we further evaluated these statin-compound combinations across a
184 large panel of 47 breast cancer cell lines. A 5-day cytotoxicity assay (sulforhodamine B assay; SRB) in a
185 6x10 dose matrix was used to assess fluvastatin-compound efficacy. As expected, dipyridamole treatment
186 resulted in a dose-dependent decrease in fluvastatin IC₅₀ value (**Supplemental Figure 6A**). Similarly,
187 nelfinavir and honokiol treatment also resulted in a dose-dependent decrease in fluvastatin IC₅₀ values

188 similar to that of dipyridamole (**Supplemental Figure 6A**). This suggests that our computational
 189 pharmacogenomic pipeline predicts compounds that potentiate statin activity similarly to dipyridamole
 190 across multiple subtypes of breast cancer cell lines.

191 Next, we evaluated statin-compound synergy using the Bliss Index model derived using
 192 SynergyFinder³³ across the panel of breast cancer cell lines. Like the dose-dependent sensitivity data, we
 193 observed that the trend in synergy between fluvastatin-dipyridamole across the 47 breast cancer cell lines
 194 was also seen with fluvastatin-nelfinavir and fluvastatin-honokiol (**Figure 4A**). Since we had previously
 195 identified that the basal subtype of breast cancer cell lines were more sensitive to single agent
 196 fluvastatin¹⁶, we evaluated whether basal breast cancer cell lines were similarly more sensitive to the
 197 fluvastatin-compound combinations. Using the SCMOD2 subtyping scheme, we evaluated the basal,
 198 HER2 and luminal B status of each cell line and determined synergy is independent of BC subtype
 199 (**Supplemental Figure 6B**). This suggests these statin-compound combinations can be applied to
 200 multiple breast cancer subtypes as therapeutic options, however a biomarker to distinguish those with
 201 high-sensitivity remained unclear.

202 Because the synergy profiles across the three fluvastatin-compound combinations were
 203 significantly similar (Fluva-NFV vs Fluva-DP, $R_s=0.55$, $p\text{-value}=7.1e-05$; Fluva-HNK vs Fluva-DP, $R_s=0.62$,
 204 $p\text{-value}=5.5e-06$; Fluva-NFV vs Fluva-HNK, $R_s=0.82$, $p\text{-value} < 2.2e-16$), we next interrogated whether
 205 baseline gene and/or protein expression profiles across the cell lines for each of the statin-compound
 206 combinations was associated with high-sensitivity and the synergistic response. To further interrogate the
 207 similarity between the statin-compound combinations, we correlated the RNA-seq and reverse phase
 208 protein array (RPPA) profiles of the 47 breast cancer cell lines³⁴ with their synergy scores for each of the
 209 statin-compound combinations. These represent the transcriptomic and proteomic state associations with
 210 synergy for each combination. We then evaluated the correlation between these associations across the
 211 different combinations (Fluva-DP vs Fluva-NFV; Fluva-DP vs Fluva-HNK; Fluva-NFV vs Fluva-HNK)
 212 (**Figure 4B**) and identified a high positive correlation between the combinations on the basis of similar
 213 transcriptomic associations (Fluva-NFV vs Fluva-DP, $R_s=0.73$, $p\text{-value} < 2.2e-16$; Fluva-HNK vs Fluva-DP,
 214 $R_s=0.77$, $p\text{-value} < 2.2e-16$; Fluva-NFV vs Fluva-HNK, $R_s=0.87$, $p\text{-value} < 2.2e-16$). This high positive
 215 correlation was also seen between these combinations using proteomic (RPPA) and synergy data

216 **(Supplemental Figure 6C)** suggesting that similar pathways were associated with the synergistic
217 response to the three statin-compound combinations.

218 To compare the overlap in pathways associated with sensitivity to fluvastatin alone, and synergy
219 between the fluvastatin-compound combinations, a Gene Set Enrichment Analysis (GSEA) using the
220 Hallmark Gene Set Collection was performed³⁵. These results showed that enriched pathways were
221 highly similar amongst fluvastatin alone and the fluvastatin-compound combinations with one of the
222 highest scoring enriched pathways being epithelial-mesenchymal transition (EMT) **(Figure 4C)**. To further
223 support this finding and because of the low agreement amongst EMT gene sets, we also evaluated four
224 additional GSEA EMT pathways and observed similar trends between fluvastatin alone and the
225 fluvastatin-compound combinations for each of the EMT gene sets **(Supplemental Figure 6D)**. As we
226 and others have published that mesenchymal-enriched cancer cell lines are more sensitive to statin
227 monotherapy^{36,37}, this data suggests that fluvastatin is the primary driver of response to these
228 statin-compound combinations. This is consistent with fluvastatin inhibiting the MVA pathway, triggering
229 the SREBP-mediated feedback response, which in turn is inhibited by the second compound
230 (dipyridamole, nelfinavir or honokiol) in these fluvastatin-compound combinations.

231 We then examined the individual genes within each of the GSEA EMT pathways to identify a
232 biomarker of the synergistic response to the statin-compound combinations. Within the EMT field, gene
233 set signatures have low agreement **(Supplemental Figure 7)**. Previously our lab published a binary
234 classifier of five EMT genes to predict increased sensitivity to statins across 631 cell lines representing
235 multiple cancer types³⁶. We evaluated whether this binary five-gene classifier could also predict synergy
236 between the different fluvastatin-compound combinations. The five-gene EMT classifier could predict
237 sensitivity to fluvastatin alone across the panel of breast cancer cell lines **(Supplemental Figure 8A)**, but
238 failed to predict synergy to the fluvastatin-compound combinations **(Supplemental Figure 8B)**. We next
239 interrogated each of the five genes individually. Interestingly, low gene expression and protein levels of
240 E-cadherin (*CDH1*), a canonical epithelial state marker, not only predicted sensitivity to fluvastatin but
241 also demonstrated synergy across all three fluvastatin-compound combinations **(Figure 5A-B and**
242 **Supplemental Figure 8C)**. To validate our findings, we probed for basal E-cadherin protein expression
243 across a panel of nine breast cancer cell lines and showed that synergy to the novel statin-compound
244 combinations is positively associated with low E-cadherin protein expression **(Figure 5C-D)**. Overall, this

data validates that our MVA-DNF pharmacogenomics strategy can successfully distinguish compounds that, like-dipyridamole, can synergize with statins to trigger BC tumour cell death.

Discussion

By blocking the statin-induced restorative feedback response, dipyridamole potentiates statin efficacy to drive tumour cell death^{21,23}. However, as the platelet-aggregation activity of dipyridamole may preclude its use in some cancer patients, our goal was to expand this class of agents that potentiate the pro-apoptotic activity of statins. To this end, we developed a novel computational pharmacogenomics pipeline that distinguished compounds that are similar to dipyridamole at the level of structure, MVA pathway gene expression perturbation, and anti-proliferative activity. We identified 23 potential dipyridamole-like compounds and then evaluated several of the top hits for their ability to phenocopy dipyridamole. By this approach, we validated that nelfinavir and honokiol sensitize breast cancer cell lines to statin-induced cell death by blocking the statin-induced restorative feedback loop. Analysis of basal RNA and protein expression identified the canonical EMT gene *CDH1* (E-cadherin) as a biomarker of the synergistic response to both statin-nelfinavir and statin-honokiol treatment. Thus, despite the polypharmacology of dipyridamole, the computational pharmacogenomics screen described here successfully identified synergistic statin-compound drug combinations as novel anti-breast cancer therapies.

By integrating a computational pharmacogenomics pipeline and cellular validation, we provide a novel, rapid, broadly-adaptable, and inexpensive strategy to distinguish compounds with similar biological activities. We show here that this approach overcomes a major problem associated with working with drugs, such as dipyridamole, that possess a complex polypharmacology. Dipyridamole was originally identified for its anti-platelet aggregation activity, but its mechanism of action spans a wide variety of functions. Several activities of dipyridamole have been described including an inhibitor of phosphodiesterases³⁸, nucleoside transport³⁹ and glucose uptake⁴⁰. By restricting the gene perturbation layer of our pharmacogenomics pipeline to MVA-pathway genes, we successfully circumvented many of these varied functions and focused specifically on identifying dipyridamole-like drugs whose mechanisms

270 centre on the mevalonate pathway. This highlights that the computational pharmacogenomics pipeline
271 described here is likely tunable to drug-specific structural features, activities and signaling pathways.

272 The new statin-sensitizing agents identified here using MVA-DNF include nelfinavir and honokiol,
273 which like dipyridamole, inhibit statin-induced SREBP2 cleavage and activation^{21,23}. To date, a number of
274 SREBP2 inhibitors have been identified that block SREBP2 processing from its precursor to mature form,
275 including fatostatin, betulin, and xanthohumol (ER-Golgi translocation), PF-429242 (site-1 protease (S1P)
276 cleavage), and nelfinavir and 1,10-phenanthroline (site-2 protease (S2P) cleavage). Additional SREBP2
277 inhibitors include BF175 and tocotrienols that target SREBP2 transcriptional activity and protein stability,
278 respectively. However, other than nelfinavir, these agents are either under development for clinical
279 application or are only used for research purposes. The S2P protease inhibitor nelfinavir (marketed as
280 Viracept) was approved for use in 1997 as an antiviral for the treatment of HIV, and is under evaluation for
281 its utility as an anti-cancer agent⁴¹⁻⁴⁶. This further reinforces that the novel combination of statin-nelfinavir
282 is immediately actionable and should be evaluated without delay. We suggest the fluvastatin-nelfinavir
283 combination is preferred compared to other statins, as distinct cytochrome P450 enzymes are used to
284 process these agents, thereby preventing adverse drug-drug interactions⁴⁷.

285 To the best of our knowledge, this is the first study to report honokiol to synergize with statins in
286 the context of cancer. Honokiol is a natural product commonly used in traditional medicine and has a
287 number of reported mechanisms of action. How honokiol inhibits SREBP2 remains unknown, but this is
288 the first study to interrogate its activity in SREBP2 translocation and gene expression alone and in
289 combination with statins. As honokiol and its derivatives are presently under development, our findings of
290 this new mechanism for honokiol can be incorporated into future analysis of honokiol's structure-activity
291 relationships. Two additional predicted dipyridamole-like compounds tested in this study include
292 selumetinib and mitoxantrone; the former was observed to sensitize breast cancer cells to statin-induced
293 apoptosis, but the latter did not. Selumetinib functions through an SREBP2-independent mechanism,
294 which suggests that not only is the identification of feedback-dependent mechanisms beneficial for cancer
295 treatment, but that additional feedback-independent classes of statin-sensitizers can be identified. This is

296 particularly relevant, as some multiple myeloma and prostate cancer cell lines have been shown to lack
297 the feedback response^{20,21,48}.

298 The data presented here have important clinical implications for statins as anti-cancer agents.
299 Despite encouraging positive results from window-of-opportunity clinical trials in breast cancer using
300 statins as a single-agent, a modest effect was observed with some but not all patients^{18,19}. Accordingly,
301 discovery of novel therapeutic combinations is necessary to achieve significant clinical impact. Since
302 nelfinavir is poised for repurposing, and statins have demonstrated anti-cancer activity in early-phase
303 clinical trials^{18,19,49-54}, clinical studies to further evaluate the therapeutic benefit of this combination could
304 proceed swiftly. Furthermore, analyses of gene and protein expression data across our large collection of
305 breast cancer cell lines identified a mesenchymal-enriched gene expression profile as highly predictive of
306 sensitivity to all three statin-compound (dipyridamole, nelfinavir or honokiol) combinations. We further
307 showed that *CDH1* expression levels served as a biomarker of synergistic response. This reinforces the
308 dipyridamole-like behaviour of nelfinavir and honokiol, identified by our pharmacogenomics pipeline, and
309 creates opportunities for biomarker-guided clinical studies (**Figure 5E**). We also observed this synergistic
310 response to the combination therapies across multiple subtypes of breast cancer. Previously we had
311 identified the basal-like breast cancer subtype as more sensitive to statins alone; here, we have
312 expanded the scope of statin treatment to encompass the wider breast cancer population which can be
313 distinguished on the basis of *CDH1* expression.

314 Collectively, our computational pharmacogenomics pipeline underscores the ability to identify
315 compounds that phenocopy our parent compound of interest (dipyridamole), importantly, in a
316 pathway-specific manner (mevalonate). Our study also provides a strong preclinical rationale to warrant
317 further investigation of the fluvastatin-nelfinavir combination, as well as the utility of *CDH1* as a biomarker
318 of response. The availability of these approved, well-tolerated drugs as well as simple methods for
319 assessing *CDH1* expression could enable rapid translation of these findings to improve breast cancer
320 outcomes.

321 **Methods**

322 Our analysis design encompasses both computational identification and refinement of dipyridamole-like
323 compounds, as well as experimental validation of the most promising candidates.

324 **MVA-specific Drug Network Fusion (MVA-DNF).**

325 We developed a computational pharmacogenomic pipeline (MVA-DNF) that facilitates identification of
326 analogues to dipyridamole, by elucidating drug-drug relationships specific to the mevalonate (MVA)
327 pathway. MVA-DNF briefly extends upon some principles of the drug network fusion algorithm we had
328 described previously⁵⁵, by utilizing the similarity network fusion algorithm across three drug taxonomies
329 (drug structures, drug perturbation, and drug sensitivity). Drug structure annotations and drug
330 perturbation signatures are obtained from the LINCS-L1000 dataset²⁶, and drug sensitivity signatures are
331 obtained from the NCI-60 drug panel²⁷. Drug structure annotations were converted into drug similarity
332 matrices by calculating tanimoto similarity measures⁵⁶ and extended connectivity fingerprints⁵⁷ across all
333 compounds, as described previously⁵⁵. We extracted calculated Z-scores from drug-dose response
334 curves for the NCI-60 drug sensitivity profiles, and computed Pearson correlation across these profiles to
335 generate a drug similarity matrix based on sensitivity²⁷. We used our PharmacGx package (version
336 1.6.1) to compute drug perturbation signatures for the L1000 dataset using a linear regression model, as
337 described previously⁵⁸. The regression model adjusts for cell specific differences, batch effects and
338 experiment duration, to generate a signature for the effect of drug concentration on the transcriptional
339 state of a cell. This facilitates identification of gene expression which has been significantly perturbed due
340 to drug treatment. These signatures indicate transcriptional changes that are induced by compounds on
341 cancer cell lines. We further refined the drug perturbation profiles to a set of six MVA-pathway genes
342 (**Supplementary Figure 1A**) that had been obtained from the literature as well as repositories of
343 pathway-specific gene sets including MSigDB⁵⁹, HumanCyc⁶⁰ and KEGG^{61,62}. These gene sets include
344 'mevalonate pathway' and 'superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)'
345 from the HumanCyc⁶³, and 'Kegg Terpenoid Backbone Biosynthesis' from KEGG^{61,62}. The filtered

346 drug-induced gene perturbation signatures were subsequently used to generate a drug perturbation
347 similarity matrix that elucidates drug-drug relationships based on common transcriptional changes across
348 the six MVA-pathway genes. We calculated similarity between estimated standardized coefficients of drug
349 perturbation signatures using the Pearson correlation coefficient. Finally, we used the similarity network
350 fusion algorithm⁶⁴ to integrate the affinity matrices for drug structure, drug sensitivity, and MVA-pathway
351 specific drug perturbation profiles, to generate an MVA-pathway specific drug taxonomy (MVA-DNF)
352 spanning 238 compounds.

353 Identification of analogues to dipyrindamole

354 We interrogated the MVA-DNF taxonomy using a variety of approaches to identify a candidate set of
355 dipyrindamole-like compounds. Using MVA-DNF similarity scores, we first generated a ranking of all
356 compounds closest to dipyrindamole. We then conducted a permutation test, to assess the statistical
357 relationship of each ranked drug against dipyrindamole. Briefly, drug fusion networks were generated 999
358 times across perturbation, sensitivity, and drug structure profiles, each time using a random set of six
359 genes to generate a 'pathway-centric' drug perturbation similarity matrix. Z-scores and p-values were
360 calculated to determine the statistical relevance of a given dipyrindamole-like analog in MVA-DNF,
361 compared to the randomly generated networks. From this, we further ranked a list of dipyrindamole-like
362 candidate compounds by their statistical significance within MVA-DNF (p-value<0.05), resulting in
363 identification of 23 candidate dipyrindamole analogs.

364 For each of the dipyrindamole analogues we identified, we conducted a similar assessment of
365 significance to identify the relationships of these compounds to dipyrindamole and to themselves. A
366 network of dipyrindamole-like analogues was rendered using the iGraph R package⁶⁵. Using MVA-DNF
367 similarity scores, we further assessed the contribution of each of the drug layers (structure, sensitivity and
368 perturbation) towards the identification of dipyrindamole-like compounds.

369 We assessed the regulation of gene expression for genes involved in the mevalonate pathway
370 across all of the top-selected dipyrindamole analogues, by analyzing the drug-induced transcriptional

profiles (described above) of the selected analogues. To further prioritize the dipyridamole analogues, the candidate compounds were categorized, and compounds that were known toxins or carcinogens were excluded from the analysis (**Supplemental Table 1, Supplemental Figure 1C**). Top hits from the largest categories were selected for further validation.

Cell culture and compounds

All cell lines were cultured as described previously^{16,23}. Briefly, MDA-MB-231 and HCC1937 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute medium (RPMI), respectively. All media was supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin. Cell lines were routinely confirmed to be mycoplasma-free using the MycoAlert Mycoplasma Detection Kit (Lonza), and their authenticity was verified by short-tandem repeat (STR) profiling at The Centre for Applied Genomics (Toronto, ON, Canada). Fluvastatin (US Biological F5277-76) was dissolved in ethanol and dipyridamole (Sigma), nelfinavir (Sigma), honokiol (Sigma), mitoxantrone (Sigma) and selumetinib (Selleckchem) were dissolved in DMSO.

Breast cancer cell lines panel

The breast cancer cell line³⁴ panel was a generous gift from Dr. Ben amin Neel. RNAseq quantification was done using Kallisto pipeline⁶⁶ using human transcriptome reference hg38.gencodeV23⁶⁷. RPPA processed data was downloaded from Marcotte et al. 2016³⁴. SCMOD2⁶⁸ breast cancer subtypes of these cell lines were obtained using the genefu R package⁶⁹.

Breast cell-line combination viability screen

We used the sulforhodamine B colorimetric (SRB) proliferation assay⁷⁰ in 96-well plates to determine the dose-response curves. To test the combinations in the panel of BC cell lines (See Breast cancer cell lines panel), the fluvastatin/dipyridamole, fluvastatin/nelfinavir and fluvastatin/honokiol drug combinations were tested in a 6x10 dose matrix format covering a range of decreasing concentrations of each drug (highest drug dose was 20 µM fluvastatin, 20 µM dipyridamole, 10 µM nelfinavir and 20 µM honokiol), along with

all their pairwise combinations, as well as the negative control (EtOH and DMSO). We subtracted the average phosphate-buffer saline (PBS) wells value from all wells and computed the standard deviation and coefficient for each replicate. All individually treated well values were normalized to the control well values. We used Prism (v8.2.0, GraphPad Software) to compute dose-response curves with a bottom constraint equal to 0.

Cell viability assays

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed as previously described²⁰. Briefly, BC cells were seeded in 750-15,000 cells/well in 96-well plates overnight, then treated in triplicate with 0-400 μ M fluvastatin for 72 hours. Half-maximal inhibitory concentrations (IC_{50}) values were computed from dose-response curves using Prism (v8.2.0, GraphPad Software) with a bottom constraint equal to 0.

Cell death assays

Cells were seeded at 2.5×10^5 cells/plates and treated the next day as indicated. After 72 hours, cells were fixed in 70% ethanol for >24 h, stained with propidium iodide and analyzed by flow cytometry for the sub-diploid (% pre-G1) DNA population as a measure of cell death as previously described²⁰.

Immunoblotting

Cell lysates were prepared by washing cells twice with cold PBS and lysing cells in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS, 1 mM EDTA, protease inhibitors) on ice for 30 min. Lysates were cleared by centrifugation and protein concentrations were determined using the Pierce 660 nm Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein were diluted in Laemmli sample buffer, boiled for 5 min and resolved by SDS-polyacrylamide gel electrophoresis. The resolved proteins were then transferred onto nitrocellulose membranes. Membranes were then blocked for 1 hr in 5% milk in tris-buffered saline/0.1 % Tween-20 (TBS-T) at room

temperature, then probed with the following primary antibodies in 5% milk/TBS-T overnight at 4 °C: SREBP-2 (1:250, BD Biosciences, 557037), p44/42 MAPK (ERK1/2) (1:1000, Cell Signaling Technology, 4695), PARP (1:1000, Cell Signaling Technology, 9542L), α -Tubulin (1:3000, Calbiochem, CP06) and E-cadherin (1:1000, Cell Signaling Technology, 3195). Primary antibodies were detected using IRDye-conjugated secondary antibodies and the Odyssey Classic Imaging System (LI-COR Biosciences). Densitometric analysis was performed using ImageJ 1.47v software.

RNA expression analyses

Total RNA was harvested from sub-confluent cells using TRIzol Reagent (Invitrogen). cDNA was synthesized from 500 ng RNA using SuperScript III (Invitrogen). Quantitative reverse transcription PCR (qRT-PCR) was performed using the ABI Prism 7900HT sequence detection system and TaqMan probes (Applied Biosystems) for *HMGCR* (Hs00168352), *HMGCS1* (Hs00266810), *INSIG1* (Hs01650979) and *RPL13A* (Hs01578913).

Drug combinations synergy analysis

Viability scores were calculated using standard pipelines from PharmacoGx R package⁵⁸ and synergy scores represented by Bliss Index were calculated using SynergyFinder R package³³. Pearson correlation coefficient was used to measure the associations between the transcriptomic and proteomic states of cell lines and the corresponding synergy scores for each of the combinations. The transcriptomic associations were then used to rank genes for GSEA⁷¹. The Hallmark gene set collection³⁵ was downloaded from MSigDB⁷². The Piano R package was used to run GSEA analysis⁷³. Other EMT related pathways, namely "GO Positive Regulation of Epithelial To Mesenchymal Transition"⁷⁴, "GO Epithelial To Mesenchymal Transition"⁷⁴, "SARRIO Epithelial Mesenchymal Transition DN"⁷⁵, and "SARRIO Epithelial Mesenchymal Transition Up"⁷⁵, were also downloaded from MSigDB for analysis.

440 **Data Access**

441 The code and associated tutorial describing how to run the analysis pipeline are publicly available on
 442 Github at: https://github.com/DGendoo/MVA_DNF. All software dependencies are available on
 443 Bioconductor (BioC) or the Comprehensive Repository R Archive Network (CRAN), and have been listed
 444 throughout the methods as applicable.

445 **Authors' contributions**

446 Experimental Design: LZP, DG, BHK, JVL, JS, DWC
 447 Acquisition of data: JVL, EB, JL, JS
 448 Experimental analysis and interpretation: JVL, LZP, BHK, DG, WB, DWC
 449 Conception and design of the pathway-centric MVA-DNF & permutation pipeline: DG, LZP, BHK, JVL, JL
 450 Development of computational methodology: BHK, DG, WB
 451 Bioinformatics data analysis and interpretation: DG, WB, BHK
 452 Code Development: DG, WB
 453 Writing, review, and/or revision of the manuscript: JVL, LZP, DWC, WB, BHK, DG
 454 Study supervision: LZP and BHK
 455 Approved the manuscript: all authors

456 **Conflict of Interest Statement**

457 DWC serves as a consultant for Agendia, Dynamo Therapeutics, AstraZeneca, Exact Sciences, GSK,
 458 Merck, Novartis, Pfizer, Puma, Roche; receives research support (to institution) from GSK, Merck and
 459 Pfizer and Roche-Genentech, and holds intellectual property as co-inventor on a patent related to
 460 biomarkers for TTK inhibitors. All other authors declare that they have no conflicts of interest.

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469 critical feedback.

470 Figure Legends

471 Fig. 1. A schematic of the mevalonate (MVA) pathway and overview of the computational

472 **pharmacogenomics workflow. (A)** In response to fluvastatin treatment (labelled with 1), MVA pathway
 473 end-product levels decrease, triggering an SREBP-mediated feedback response that activates MVA
 474 pathway-associated gene expression to restore cholesterol and other non-sterol end-product levels.
 475 Dipyridamole (DP) (labelled with 2) blocks the SREBP-mediated feedback response, thereby potentiating
 476 fluvastatin-induced cancer cell death. **(B)** An overview of the computational pharmacogenomics workflow,
 477 MVA-DNF, used to identify the top 23 "dipyridamole-like" candidates and visualized as a compound
 478 network. MVA-DNF combines drug structure, drug sensitivity, and drug-induced gene perturbation
 479 datasets restricted to six MVA pathway-specific genes. Permutation specificity testing was performed to
 480 select compounds that have a degree of specificity to the mevalonate pathway and dipyridamole.
 481 Statistical significance of compounds similar to dipyridamole was assessed by comparing to 999 networks
 482 generated from random selection of six genes within the drug perturbation layer. A network representation
 483 of dipyridamole and top 23 statistically-significant (p -value <0.05) "dipyridamole-like" compounds are
 484 shown. Each node represents a compound and edges connect compounds based on statistical
 485 significance of p -value <0.01 . Darker blue nodes and orange edges represent the compounds connected
 486 to dipyridamole, and edge thickness represents the associated p -value between the compounds. **(C)**
 487 Radar plot of the top 23 dipyridamole-like compounds (p -value <0.05), where the contribution of each
 488 individual layer of the MVA-DNF (drug structure, sensitivity, and perturbation) is depicted. Percent
 489 contribution of each layer is shown from the center (0%) to the outer edges (100%).

490 Fig. S1, related to Fig. 1. Additional information regarding drug-induced genotype changes and

491 **categorization of top 23 dipyridamole-like compounds. (A)** Simplified schematic of the MVA pathway,
 492 highlighting the six MVA-pathway genes (in red) in the L1000 database used to restrict the drug-induced
 493 gene perturbation layer of the MVA-DNF method. **(B)** Drug perturbation signatures for dipyridamole and
 494 dipyridamole-like compounds, plotted for genes pertaining to the MVA pathway. Similarity between

495 compounds based on their overall expression profiles is rendered in the dendrogram. Dipyridamole- and
496 fluvastatin-induced changes shown on the bottom as reference. **(C)** Categorization of the top 21
497 dipyridamole-like compounds excluding toxins and carcinogenic compounds.

498 Fig. S2. MVA-DNF drug-dose response curves for MDA-MB-231 and HCC1937 breast cancer cell
499 lines to identify a sub-lethal dose of top dipyridamole-like compounds. (A) MDA-MB-231 and **(B)**
500 HCC1937 cells were treated with a range of doses for 72 hours, and cell viability was determined using
501 an MTT assay. The drug dose-response curves are plotted with a dashed line at 80% MTT activity
502 indicating a sub-lethal drug dose. Data for an average of three technical replicates are plotted; data reflect
503 the results of a single biological experiment. **(C)** Table of sub-lethal drug dose and interpolated % MTT
504 activity for both MDA-MB-231 and HCC1937.

505 Fig. S3. MVA-DNF drug-dose response curves, fluvastatin IC₅₀ and solvent control values for
506 MDA-MB-231 cells. MDA-MB-231 cells were treated with a range of fluvastatin doses alone or in
507 combination with a sub-lethal dose of dipyridamole (5 μ M), selumetinib (0.4 μ M), nelfinavir (3 μ M),
508 mitoxantrone (0.01 μ M) or honokiol (12 μ M) for 72 hours, and cell viability was determined using an MTT
509 assay. The drug dose-response curves, fluvastatin IC₅₀ values and control values are plotted. Error bars
510 represent the mean $\pm$ SD, n = 3-5, *p < 0.05, **p < 0.01 (Student *t* test, unpaired, two-tailed).

511 Fig. S4. MVA-DNF drug-dose response curves, fluvastatin IC₅₀ and solvent control values for
512 HCC1937 cells. HCC1937 cells were treated with a range of fluvastatin doses alone or in combination
513 with a sub-lethal dose of dipyridamole (5 μ M), selumetinib (1 μ M), nelfinavir (3 μ M), mitoxantrone (0.001
514 μ M) or honokiol (10 μ M) for 72 hours, and cell viability was determined using an MTT assay. The drug
515 dose-response curves, fluvastatin IC₅₀ values and control values are plotted. Error bars represent the
516 mean $\pm$ SD, n = 3-6, *p < 0.05, **p < 0.01, ***p < 0.001 (Student *t* test, unpaired, two-tailed).

Fig. 2. Dipyridamole-like compounds potentiate fluvastatin-induced cell death. (A) MDA-MB-231 and HCC1937 cells were treated with solvent controls or fluvastatin +/- dipyridamole (DP), nelfinavir (NFV), honokiol (HNK) or selumetinib (Selu) for 72 hours, fixed in ethanol and assayed for DNA fragmentation (% pre-G1 population) as a marker of cell death by propidium iodide staining. Error bars represent the mean +/- SD, $n = 3-4$, $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$ (one-way ANOVA with Bonferroni's multiple comparisons test, where each treatment was compared to the solvent control). (B) Cells were treated as in (A), protein isolated and immunoblotting was performed to assay for PARP cleavage. (F) represents full-length PARP and (C) represents cleaved PARP. (C) PARP cleavage (cleaved/full-length) shown in (B) was quantified by densitometry and normalized to Tubulin expression. Error bars represent the mean +/- SD, $n = 3-5$, $*p < 0.05$, $**p < 0.005$, $***p < 0.001$, $****p < 0.0001$ (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent control within each experiment).

Fig. 3. Nelfinavir and Honokiol block fluvastatin-induced SREBP activation. (A) MDA-MB-231 and HCC1937 cells were exposed to solvent controls, fluvastatin (Flu) +/- dipyridamole (DP), nelfinavir (NFV), honokiol (HNK) or selumetinib (Selu) for 16 hours, and RNA was isolated to assay *INSIG1* expression by qRT-PCR. mRNA expression data are normalized to *RPL13A* expression. Error bars represent the mean +/- SD, $n = 3-4$, $*p < 0.05$, $**p < 0.005$, $***p < 0.001$, $****p < 0.0001$ (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent control group within each experiment). (B) MDA-MB-231 and HCC1937 cells were treated with fluvastatin +/- dipyridamole, nelfinavir, honokiol or selumetinib for 12 hours, and protein was harvested to assay for SREBP2 expression and cleavage (activation) by immunoblotting. (P) represents precursor, full-length SREBP2 and (M) represents mature, cleaved SREBP2. (C) SREBP2 cleavage (cleaved/full-length) was quantified by densitometry and normalized to total ERK expression. Error bars represent the mean +/- SD, $n = 3-8$, $*p < 0.05$, $**p < 0.005$, $***p < 0.001$, $****p < 0.0001$ (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent controls group within its experiment).

Fig. S5, related to Fig 3. Nelfinavir and Honokiol block fluvastatin-induced SREBP activation of SREBP2 feedback genes. (A) MDA-MB-231 cells were treated with fluvastatin +/- dipyridamole, nelfinavir, honokiol or selumetinib for 16 hours, and RNA was isolated to assay for *HMGCR* and *HMGCS1* expression by qRT-PCR. mRNA expression data are normalized to *RPL13A* expression. (B) RPL13A Ct mean values plotted as a control. Error bars represent the mean +/- SD, n = 3-4, *p < 0.05, **p<0.005, ***p<0.001, ****p<0.0001 (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent controls group).

Fig. S6, related to Fig 4. High-throughput compound combination screen. (A) Heatmap of Log₁₀(Fluvastatin IC₅₀) values for a high-throughput compound synergy screen against 47 BC cell lines visualizing the 15th to 85th percentile. BC cell lines were treated with a dose matrix of fluvastatin (0-20 µM) +/- dipyridamole (DP) (0-20 µM), nelfinavir (NFV) (0-10 µM) or honokiol (HNK) (0-20 µM). After 5 days of drug treatment, cell viability was assessed by SRB assay. SCMOD2 cell line subtyping was assigned to the BC cell line panel. Data presented are the average of 2 biological replicates (fluvastatin +/- dipyridamole (DP)) or the mean of 3-6 biological replicates (fluvastatin +/- nelfinavir (NFV) and fluvastatin +/- honokiol (HNK)). (B) Comparison of synergy scores stratified by BC subtypes across the combinations using wilcoxon paired rank test. Red dash line at synergy threshold where >0 indicates lower synergy and <0 indicates higher synergy. (C) Associations of proteomic states³⁴ with synergy scores across the fluvastatin-compound combinations. Similarity of proteomic states associations were compared across the combinations (Fluva-DP vs Fluva-NFV; Fluva-DP vs Fluva-HNK; Fluva-NFV vs Fluva-HNK) using Pearson correlation coefficient. Top five basally-expressed proteins associated with synergy in either direction are annotated in red. (D) Gene set enrichment analysis using five EMT gene set collections and genes ranked by basal mRNA correlated to the fluvastatin IC₅₀ (Fluva) value or synergy score (Fluva-DP, Fluva-NFV and Fluva-HNK). Dot size indicates the difference in enrichment scores (ES) of the pathways. Background shading indicates the FDR. X indicates pathway and drug combinations that were not significantly enriched (FDR > 0.05).

Fig. 4. Compound combination synergy analysis. (A) Heatmap of synergy scores (Bliss Index model) for fluvastatin (Fluva) + dipyridamole (DP), nelfinavir (NFV) or honokiol (HNK) in a panel of 47 breast cancer cells lines. Ordered by synergy score of Fluva-DP, from greatest (<0) to least synergy (>0). Breast cancer subtype of each cell line is shown and is based on the SCMOD2 subtyping scheme. **(B)** Basal mRNA expression³⁴ associations with synergy scores for each drug combination (e.g. Fluva-NFV vs. Fluva-DP, Fluva-HNK vs. Fluva-DP, and Fluva-NFV vs. Fluva-HNK). Associations were calculated using Pearson correlation coefficient. Top five basally-expressed genes associated with synergy in either direction are annotated in red. **(C)** Gene set enrichment analysis (GSEA) using the Hallmark gene set collection, where genes were ranked according to their correlation to the fluvastatin IC₅₀ (Fluva) value or to the synergy score (Fluva-DP, Fluva-NFV and Fluva-HNK). Dot plot was restricted to pathways enriched in two out of the four groups. Dot size indicates the difference in enrichment scores (ES) of the pathways. Background shading indicates the FDR. X indicates pathway and drug combinations that were not significantly enriched (FDR > 0.05).

Fig. S7, related to Fig 4. Overlapping genes within the EMT gene sets. (A) Upset plot to visualize the agreement between Yu *et al.* (2018)³⁶ five-gene classifier and five additional EMT gene sets.

Fig. S8, related to Fig 5. EMT gene expression as a biomarker of sensitivity to fluvastatin and synergistic response to fluvastatin-compound combinations. (A) Five-gene fluvastatin sensitivity gene classifier³⁶ predicts sensitivity to fluvastatin alone, but **(B)** does not predict synergy to Fluva-DP, Fluva-NFV or Fluva-HNK. **(C)** Basal Vimentin (VIM), N-Cadherin (CDH2), ZEB1 and fibronectin (FN1) mRNA expression do not predict synergy to the drug combinations.

Fig. 5. Basal E-cadherin predicts synergistic response to fluvastatin-compound combinations. (A) Basal E-cadherin mRNA expression between cell lines predicted to be synergistic or not to each drug combination. Synergy was defined by Bliss Index and significance was measured by wilcoxon rank sum test. **(B)** Basal E-cadherin mRNA expression between cell lines predicted to be respondent or not to

591 fluvastatin. Sensitivity was defined by IC_{50} and significance was measured by wilcoxon rank sum test. **(C)**
 592 Protein lysates were isolated from a panel of breast cancer cell lines to assay for basal E-cadherin
 593 expression by immunoblotting. **(D)** Densitometry values of normalized E-cadherin expression plotted as a
 594 heatmap. E-cadherin expression was quantified by densitometry and normalized individually to Tubulin
 595 expression. **(E)** Schematic diagram detailing the potential for fluvastatin (labelled with 1) and nelfinavir
 596 (labelled with 2) to block the SREBP2-mediated feedback response and synergize to potentiate
 597 fluvastatin-induced cell death.

598 Table Legends

599 Supplementary Table 1 - Ranked MVA-DNF compounds. Z-score and p-values are indicated.
 600 Compounds are ordered by p-value.

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FIGURE 1

SUPP FIGURE 1

A

B

C

C

	MDA-MB-231		HCC1937	
Drug	Dose	Interpolated MTT activity	Dose	Interpolated MTT activity
Selumetinib	0.4 μM	91.92%	1 μM	83.97%
Nelfinavir	3 μM	114.8%	3 μM	91.8%
Mitoxantrone	0.01 μM	~80%	0.001 μM	85.4%
Honokiol	12 μM	102.99%	10 μM	92.97%

SUPP FIGURE 3 (MDA-MB-231)

SUPP FIGURE 4 (HCC1937)

FIGURE 2

A

SUPP FIGURE 5

A

B

SUPP FIG 6

FIGURE 4

A

B

C

SUPP FIG 7

FIGURE 5

A

B

C

D

E

SUPP TABLE 1

Hits	Permutation Test		Currently used in humans	Currently used in cancer treatment	In clinical trials for cancer treatment	Pre-clinical	Research tool	Exclude	Classification
	Z-Score	P-Value*							
SELUMETINIB	-3.57	1.77E-04	+		+				RAF/MEK inhibitor
NELFINAVIR	-3.28	5.18E-04	+		+				Antiretroviral
MITOXANTRONE	-3.20	6.82E-04	+	+	+				Anthracycline
DOXORUBICIN	-3.03	1.22E-03	+	+	+				Anthracycline
HONOKIOL	-2.96	1.53E-03				+			Natural product
CLOTRIMAZOLE	-2.95	1.57E-03	+						Antifungal
SULFATHIAZOLE	-2.89	1.90E-03				+			Antibiotic
VEMURAFENIB	-2.67	3.79E-03	+	+	+				RAF/MEK inhibitor
CHROMOMYCINA3	-2.64	4.13E-03				+	+	Toxin	Antibiotic
BACCATINIII	-2.53	5.69E-03				+			Natural product
NOSCAPINE	-2.51	6.11E-03			+	+			Natural product
METHOTREXATE	-2.29	1.11E-02	+	+	+				Antimetabolite
CADMIUMCHLORIDE	-2.22	1.33E-02				+	+	Carcinogen	Other
RHAMNETIN	-2.18	1.45E-02				+			Natural product
PENTAMIDINE	-2.18	1.46E-02	+		+				Antifungal
EMODIN	-2.16	1.55E-02				+			Natural product
FLUOROURACIL	-2.11	1.75E-02	+	+	+				Antimetabolite
TRYPTOPHAN	-1.92	2.76E-02	+		+				Other
ALVOCIDIB	-1.82	3.44E-02	+	+	+				CDK inhibitor
ABIRATERONE	-1.77	3.81E-02	+	+	+				Antiandrogen
THAPSIGARGIN	-1.71	4.36E-02				+			Other
KINETINRIBOSIDE	-1.66	4.84E-02				+			Other
ELLIPTICINE	-1.65	4.92E-02				+			Natural product

* Table ordered by p-value

Figures

Figure 1

A schematic of the mevalonate (MVA) pathway and overview of the computational pharmacogenomics (A) In response to fluvastatin treatment (labelled with 1), MVA pathway end-product levels decrease, triggering an SREBP-mediated feedback response that activates MVA pathway-associated gene expression to restore cholesterol and other non-sterol end-product Dipyridamole (DP) (labelled with 2) blocks the SREBP-mediated feedback response, thereby potentiating fluvastatin-induced cancer cell (B) An overview of the computational pharmacogenomics workflow, MVA-DNF, used to identify the top 23 "dipyridamole-like" candidates and visualized as a compound MVA-DNF combines drug structure, drug sensitivity, and drug-induced gene perturbation datasets restricted to six MVA pathway-specific Permutation specificity testing was performed to select compounds that have a degree of specificity to

the mevalonate pathway and Statistical significance of compounds similar to dipyridamole was assessed by comparing to 999 networks generated from random selection of six genes within the drug perturbation A network representation of dipyridamole and top 23 statistically-significant (p-value <0.05) "dipyridamole-like" compounds are Each node represents a compound and edges connect compounds based on statistical significance of p-value <0.01. Darker blue nodes and orange edges represent the compounds connected to dipyridamole, and edge thickness represents the associated p-value between the (C) Radar plot of the top 23 dipyridamole-like compounds (p-value <0.05), where the contribution of each individual layer of the MVA-DNF (drug structure, sensitivity, and perturbation) is Percent contribution of each layer is shown from the center (0%) to the outer edges (100%).

Figure 2

Dipyridamole-like compounds potentiate fluvastatin-induced cell (A) MDA-MB-231 and HCC1937 cells were treated with solvent controls or fluvastatin +/- dipyridamole (DP), nelfinavir (NFV), honokiol (HNK) or selumetinib (Selu) for 72 hours, fixed in ethanol and assayed for DNA fragmentation (% pre-G1 population) as a marker of cell death by propidium iodide Error bars represent the mean +/- SD, n = 3-4, *p < 05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Bonferroni's multiple comparisons test, where each

treatment was compared to the solvent control). (B) Cells were treated as in (A), protein isolated and immunoblotting was performed to assay for PARP (F) represents full-length PARP and (C) represents cleaved PARP. (C) PARP cleavage (cleaved/full-length) shown in (B) was quantified by densitometry and normalized to Tubulin Error bars represent the mean +/- SD, n = 3-5, *p < 0.05, **p<0.005, ***p<0.001, ****p<0.0001 (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent control within each experiment).

Figure 3

Nelfinavir and Honokiol block fluvastatin-induced SREBP (A) MDA-MB-231 and HCC1937 cells were exposed to solvent controls, fluvastatin (Flu) +/- dipyridamole (DP), nelfinavir (NFV), honokiol (HNK) or selumetinib (Selu) for 16 hours, and RNA was isolated to assay INSIG1 expression by qRT-PCR. mRNA expression data are normalized to RPL13A Error bars represent the mean +/- SD, n = 3-4, *p < 0.05, **p<0.005, ***p<0.001, ****p<0.0001 (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent control group within each experiment). (B) MDA-MB-231 and HCC1937 cells were treated with fluvastatin +/- dipyridamole, nelfinavir, honokiol or selumetinib for 12 hours, and protein was harvested to assay for SREBP2 expression and cleavage (activation) by (P) represents precursor, full-length SREBP2 and (M) represents mature, cleaved (C) SREBP2 cleavage (cleaved/full-length) was quantified by densitometry and normalized to total ERK Error bars represent the mean +/- SD, n = 3-8, *p < 0.05, **p<0.005, ***p<0.001, ****p<0.0001 (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent controls group within its experiment).

Figure 4

Compound combination synergy (A) Heatmap of synergy scores (Bliss Index model) for fluvastatin (Fluva) + dipyrindamole (DP), nelfinavir (NFV) or honokiol (HNK) in a panel of 47 breast cancer cells Ordered by synergy score of Fluva-DP, from greatest (<0) to least synergy (>0). Breast cancer subtype of each cell line is shown and is based on the SCMOD2 subtyping (B) Basal mRNA expression³⁴ associations with synergy scores for each drug combination (e.g. Fluva-NFV Fluva-DP, Fluva-HNK Fluva-DP, and Fluva-NFV vs. Fluva-HNK). Associations were calculated using Pearson correlation Top five basally-expressed genes associated with synergy in either direction are annotated in (C) Gene set enrichment analysis (GSEA) using the Hallmark gene set collection, where genes were ranked according to their correlation to the fluvastatin IC₅₀ (Fluva) value or to the synergy score (Fluva-DP, Fluva-NFV and Fluva-HNK). Dot plot was restricted to pathways enriched in two out of the four groups. Dot size indicates

the difference in enrichment scores (ES) of the pathways. Background shading indicates the X indicates pathway and drug combinations that were not significantly enriched (FDR > 05).

Figure 5

Basal E-cadherin predicts synergistic response to fluvastatin-compound combinations. (A) Basal E-cadherin mRNA expression between cell lines predicted to be synergistic or not to each drug Synergy was defined by Bliss Index and significance was measured by wilcoxon rank sum (B) Basal E-cadherin mRNA

expression between cell lines predicted to be respondent or not to sensitivity was defined by IC50 and significance was measured by wilcoxon rank sum test. (C) Protein lysates were isolated from a panel of breast cancer cell lines to assay for basal E-cadherin expression by (D) Densitometry values of normalized E-cadherin expression plotted as a E-cadherin expression was quantified by densitometry and normalized individually to Tubulin (E) Schematic diagram detailing the potential for fluvastatin (labelled with 1) and nelfinavir (labelled with 2) to block the SREBP2-mediated feedback response and synergize to potentiate fluvastatin-induced cell