

1 Personalized anti-cancer drug combination prediction by an Integrated Multi-level

2 Network

3 Running title: Network-based prediction of anti-cancer drug combination

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21 **Abstract**

22 Anti-cancer drug combination is an effective solution to improve treatment efficacy and
23 overcome resistance. Here we propose a network-based method (DComboNet) to prioritize the
24 candidate drug combinations. The level one model is to predict generalized anti-cancer drug
25 combination effectiveness and level two model is to predict personalized drug combinations. By
26 integrating drugs, genes, pathways and their associations, DComboNet achieves better performance
27 than previous methods, with high AUC value of around 0.8. The level two model performs better
28 than level one model by introducing cancer sample specific transcriptome data into network
29 construction. DComboNet is further applied on finding combinable drugs for sorafenib in

30 hepatocellular cancer, and the results are verified with literatures and cell line experiments. More
31 importantly, three potential mechanism modes of combinations were inferred based on network
32 analysis. In summary, DComboNet is valuable for prioritizing drug combination and the network
33 model may facilitate the understanding of the combination mechanisms.

34 *Keyword*

35 Cancer, Drug Combination, Multi-level Heterogeneous Network, Drug Induced
36 Transcriptomic Changes

37
38

39 **Background**

40 Cancer is the leading life-threatening disease across the world with more than eighteen
41 million new diagnosed cancer cases and 9.6 million death in 2018 [1]. Due to the genetic and
42 phenotypic heterogeneity of cancer, conventional anti-cancer monotherapies could not reach
43 the expectation of clinical outcome. Unavoidable resistance and side effects induced by some
44 monotherapies require effort on exploring more effective treatment strategies. Therefore,
45 combining anti-cancer medicines has become a feasible alternative because of their advantages
46 on sensitizing cancer response, modulating multiple biological progresses or pathways and
47 reducing side effects[2]. Till 2015, only 49 anti-cancer combinatorial chemotherapies have been
48 approved by FDA [3]. To discover more drug combinations, several high-throughput drug
49 combination screening on cancer cell lines have been established which allow hundreds of drug

50 pairs being tested in short time [4]. However, experimental screening all anti-cancer drug pairs
51 exhaustively is impractical. Thus, in-silico discovery of potential drug combinations is
52 considered as a reasonable way.

53 Two major strategies are considered for constructing more precise prediction models, that is
54 to predict whether drugs can combine to achieve synergism and if the combinations can
55 combine in a certain disease context. To address the former questions, methods like Zhao's
56 integrated drug-drug similarities including drug indication, drug ATC code, drug target proteins
57 and drug side effects to predict effective drug combinations[5]. With the accumulation of cancer
58 sample/cell lines transcriptome data and the understanding of molecular mechanism between
59 drug and cancer, drug combination prediction in the context of cancer sample has gradually
60 become the main direction. Databases like CCLE and LINCS released drug treated cancer cell
61 line transcriptome data offer a solid base to support the construction of a cancer-specific
62 dynamic network which reflect the real drug function [6-8]. Dialogue for Reverse Engineering
63 Assessments and Methods consortium (DREAM) launched a worldwide open challenge in
64 2014 for drug synergy/combination prediction aimed at developing prediction models based on
65 the integration of multilevel data [9]. Among the 31 submitted models, DIGRE, the top one
66 algorithm, predict drug synergy based on modelling drug combination induced transcriptome
67 changes from monotherapy perturbations[10]. SynGen predict combinable drug pairs which
68 work complementary towards master regulators who induce cell death or inhibiting cell status
69 activation [9]. Following the challenge, Cao et al, proposed a well performed model compared
70 with other methods based on semi-supervised learning called RACS[11]. It integrated seven
71 features from drug targeting networks and two filtering parameters from transcriptomic profiles

72 and predicted potential drug combination based on the similarities to positive dataset. Though
73 the features in RACS and other models were more focusing on local similarity between drug
74 targets or the function of targets, the integration of multi-level drug related information and the
75 combination of targeting network and drug treatment transcriptome profile provide a new
76 notion of building a dynamic disease network interpreted by drug treatment. With the
77 accumulation of high-throughput drug screening data, several supervised models have been
78 built based on large high-throughput drug synergy screen dataset, like 39 drugs for 38 cancer
79 cell lines provided by O'Neil [12] and 710 drug combinations across 85 cancer cell lines in
80 drug synergy prediction DREAM challenge [13]. Algorithms like DMIS, NAD and Y Guan,
81 performed in top three position in DREAM challenge, predict drug synergism on cell lines via
82 multi-dimensional feature extraction and machine learning methods[13]. These algorithms
83 showed good performance on the data set provided by DREAM[13]. Later on, deep learning
84 model Deepsynergy used similar strategy achieve good performance[14]. However, these
85 supervised learning algorithms tend to have high dependence on a large number of cell line
86 experimental data to achieve good predictions on the corresponding cell lines. Furthermore, the
87 algorithms are usually difficult to apply on other cell lines than the modelling set. When tested
88 on the O'Neil data set, the performance of Y Guan, DMIS, NAD are all dropped[13]. Although
89 performed well on cross-validation on modelling dataset, Deepsynergy did not verify in
90 external dataset[14].

91 The complexity of drug mechanism on real cancer context is still a main obstacle in
92 combination prediction. Constructing a heterogeneous network is an applicable solution for
93 integrating multilevel information and modeling different biological systems[15, 16]. Han's

94 method mapped drug on gene expression profile weighted PPI network via drug-target
95 associations to predict drug-drug interactions [17]. WNS method evaluated drug synergy in
96 pathway-pathway interaction network [18]. DrugComboRanker discovered potential drug
97 combination by identifying drug-drug associations in target networks[19]. Barabasi's synergy
98 prediction is based on measuring the distance between drug modules and disease modules
99 discovered from the gene network between drug targets and disease related genes[20]. In
100 addition to use in predicting synergistic drug combinations, network-based approaches may
101 help infer potential mechanism between combinable drug pairs via the construction of
102 biological network[15]. However, these network-based methods based mainly on drug and their
103 known direct target genes, even with the integration of pre-treatment transcriptome data, they
104 did not show enough power to predict sample-specific drug combinations.

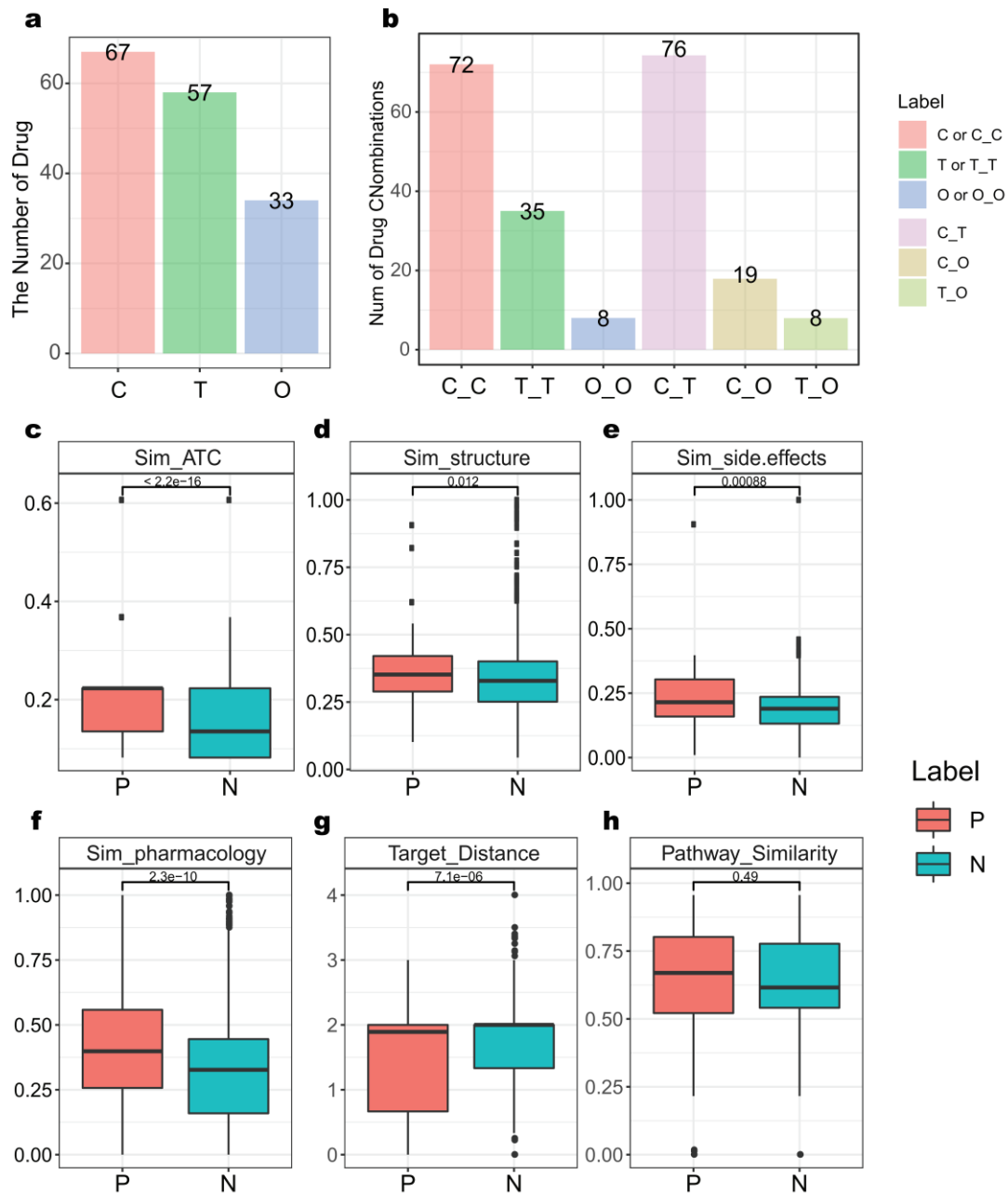
105 Assuming pharmacologically similar and functionally related drugs tend to combine together,
106 we proposed a computational method called DComboNet to predict the anti-cancer drug
107 combination. The DComboNet level one model constructs a generalized heterogeneous
108 network integrating drug-drug, drug-gene, drug-pathway, gene-gene and pathway-pathway
109 associations. Drugs that can be combined with the drug seed are predicted according to their
110 global similarity in the network. The level two model constructs a cancer sample specific
111 network to predict personalized drug combination. DComboNet was evaluated using cross
112 validation, independent test and experimental validation. DComboNet outperformed the
113 previous methods. Additionally, DComboNet provides clues for the potential mechanisms of
114 drug combinations.

115

116 **Results**

117 **Characteristics of know anti-cancer drug combinations**

118 We collected 218 known anti-cancer drug combinations that involved in 157 drugs. There were
119 three types of drugs: 67 standard chemotherapy (C), 57 targeted cancer therapy (T) and 33 other
120 kind of drugs (O) (**Fig. 1a**). The effects and anti-tumor mechanisms of these three types drugs are
121 distinctive. Standard chemotherapy acts on both normal and cancerous cells via their cytotoxic
122 function; targeted cancer therapies are deliberately chosen or designed to interact with their
123 specific target or targets with a cytostatic mechanism; other drugs may help control cancer related
124 complications or alleviate on side effects caused by anti-cancer medicine. The combinations
125 within and between three types can all be seen in known anti-cancer drug combination (**Fig. 1b**).



126

127 **Figure 1.** Features of known anti-cancer drug combinations. **a** The distribution of drug types. C, T and O are
 128 standard chemotherapy, targeted cancer therapy and other cancer-related drugs, respectively. **b** The distribution of
 129 drug combinations by drug types. **c-e** Comparison of ATC code similarity, chemical structural similarity and side-
 130 effect similarity between known drug combinations (P) and unlabeled drug pairs (N); **f-h** Comparison of the
 131 integrated pharmacological similarity, target distance and pathway similarity between known anti-cancer drug
 132 combinations (P) and unlabeled drug pairs (N).

133

134 The mechanisms of drug combinations can be partially explained by pharmacological similarity,

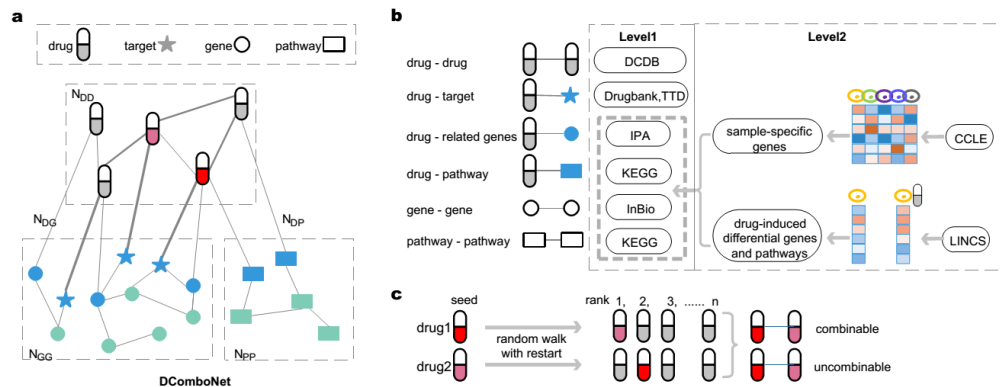
135 topological associations of drug targeted genes and functional pathways[5, 11, 18]. To understand

136 the contribution of these features to anti-cancer drug combinations, we generated 9017 unlabeled

137 drug pairs by randomly pairing the drugs in the positive dataset and then compared unlabeled pairs
138 with known combinations. The ATC code, chemical structure fingerprints and side effects were used
139 to calculate the similarity between drug pairs respectively (**Supplementary methods**), and then
140 combined into an integrated pharmacological similarity. All the single and integrated
141 pharmacological similarity were higher in known combinations than in unlabeled pairs (**Fig.1c-f**).
142 Target distance was the average distance between two target gene sets on the background gene-gene
143 interaction network. Drugs in known combinations had shorter target distance than in unlabeled
144 pairs (**Fig. 1g**). Pathway similarity between drugs was implemented via computing the average
145 shortest distance between pathways, and if drugs co-regulate same pathway, using the shortest
146 distance between their targeted genes to represent their pathway similarity. (**Supplementary**
147 **methods**). The pathway similarity of drugs in known combinations is also higher than randomized
148 pairs, though not significant (**Fig1h**). Therefore, we thought integrating both drug pharmacological
149 and functional associations may help predict combinable drug pairs and reveal the potential
150 mechanisms.

151 **Workflow of cancer drug combination network (DComboNet)**

152 The concept of DComboNet is to abstract pharmacological and functional relationships
153 between drugs into a heterogeneous cancer drug combination network (**Fig 2a**). DComboNet
154 consists of five subnetworks: drug-drug association network (N_{DD}), drug-gene association
155 network (N_{DG}), gene-gene association network (N_{GG}), drug-pathway association network (N_{DP})
156 and pathway-pathway association network (N_{PP}).



157
 158 **Figure 2.** Workflow of drug combination prediction model (DComboNet). **a** Construction of heterogeneous cancer
 159 drug combination network (DComboNet). The network contains five sub-networks, N_{DD} indicates drug-drug
 160 association network, N_{DG} indicates drug-gene association network, N_{GG} indicates gene-gene association
 161 network, N_{DP} indicates drug-pathway association network and N_{PP} indicates pathway-pathway association
 162 network. **b** Source and methods for generating network edges. Level one indicates the generalized model and level
 163 two indicates cancer sample specific model. **c** Method of ranking drug pairs.

164

165 DComboNet contains two levels of models (**Fig. 2b**). Level one is a general model that
 166 predict the potential drug combinations. It was established without considering individual
 167 heterogeneity. Edges were generated from multiple databases and the weights of edges were
 168 assigned based on the edge types (seeing **Methods**). Level one model may be not precise enough
 169 for specific cancer type or individual sample. Introducing transcriptome data into drug
 170 combination prediction can enhance the precision of the prediction for certain cancer type [10].
 171 Therefore, level two model utilized transcriptome data to reconstruct networks and predict drug
 172 combinations for a specific cancer sample. Sample specific expressed genes were obtained by
 173 comparing the expression profile of this sample with other cancer samples. Drug induced
 174 differentially expressed genes and pathways were obtained by comparing the expression
 175 profiles before and after drug treatment.

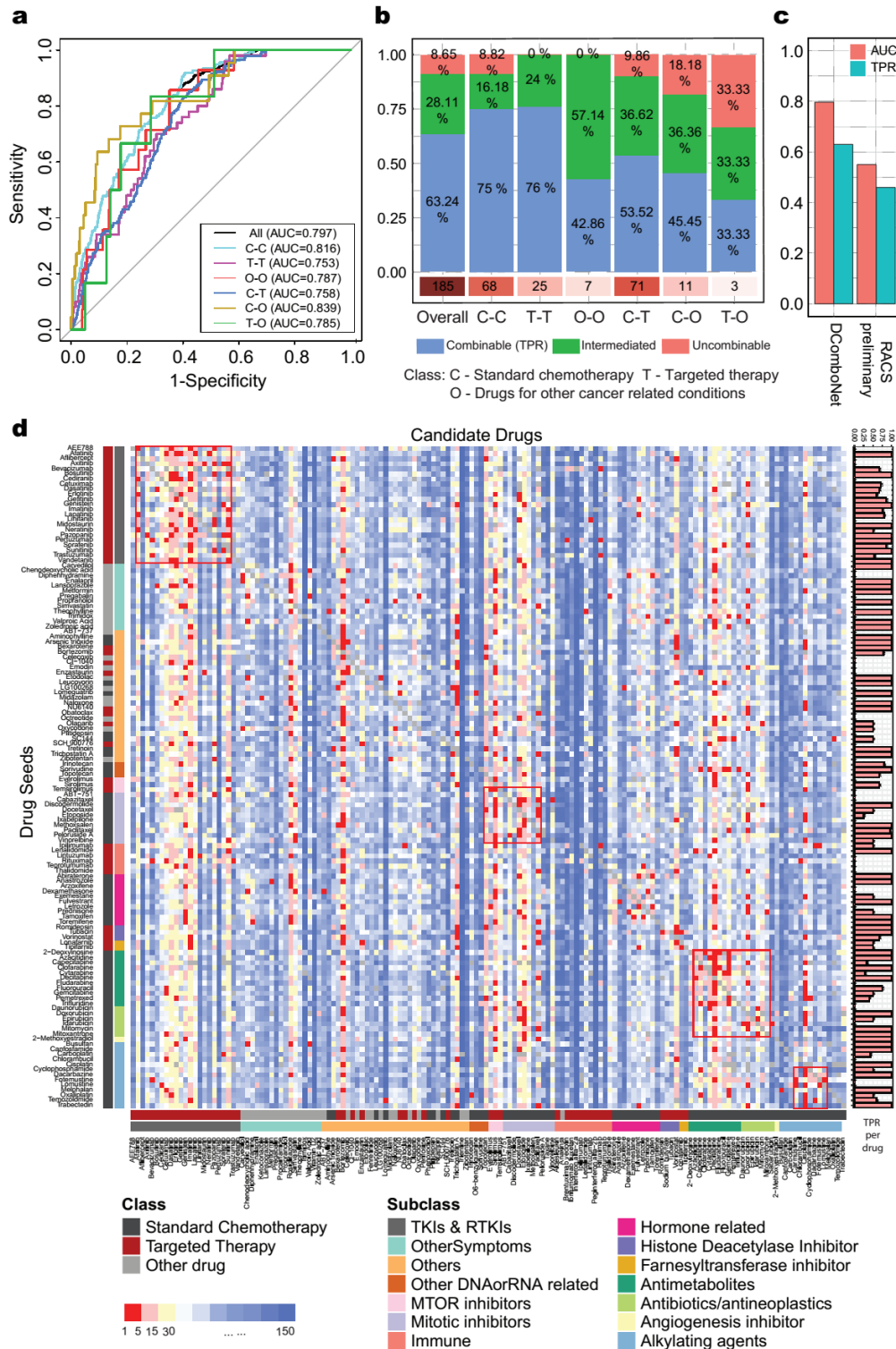
176 After network construction, Random Walk with Restart method was applied to capture the
 177 global proximity between the given drug seed and candidate drugs in the network. For a drug
 178 pair, drug1 and drug2, take each of them as seed to calculate the global proximity between drug1

179 and drug2, respectively. Then a two-threshold strategy was used to integrate two ranks and
180 classify the drug pair into combinable, uncombinable and intermediate (**Supplementary**
181 **methods**).

182

183 **Performance of level one model**

184 Leave-one-combination-out cross validation (LOCOCV) was used to evaluate the performance
185 of level one model. Firstly, we compared the prediction performance of using drug-drug network
186 alone and using the integration of multiple different networks. DComboNet integrated five
187 subnetworks and obtained the best performance (**Fig.s1c**). The AUC of DComboNet is 0.797 and
188 the true positive rate (TPR) is 63.24%. Secondly, we compared the prediction results of different
189 drug types (**Fig. 3 a-b**). Standard Chemotherapy combinations (C-C) performed well with AUC
190 equals to 0.816. All 68 real drug combinations within this category were ranked in top 50%, of
191 which 51 known combinations were predicted to as combinable. Targeted therapy drug
192 combinations (T-T) also achieved high accuracy with 17 out of 23 known combinations were
193 predicted correctly (TPR = 76%). Due to the lack of pharmacological and functional associations
194 between standard chemotherapy and targeted therapy, the accuracy of C-T combinations is slightly
195 less powerful (TPR = 53.52%). Lastly, we compared DComboNet with a previous published
196 algorithm, RACS preliminary model, which also predict without using transcriptome data [11].
197 DComboNet outperformance RACS which has AUC equal of 0.548 and true positive rate 46.0%
198 (**Fig. 3c**).



199

200 **Figure 3.** Performance of the level one model. **a** ROC plots and AUC values based on different types of drug
 201 combinations. **b** The percentage of predicted as combinable, uncombinable and intermediate in known drug
 202 combinations with respect to different drug combination types. **c** Comparison of performance between DComboNet
 203 and RACS preliminary. **d** The result of Level one model DComboNet. Heatmap was sorted in two directions
 204 according to drug subclasses (Table s1). Each row represents a drug seed. Color in the plot shows the rank of
 205 similarity between drug seed and other drugs. The bar plot on the right shows the successfully predicted drug pairs

206 in known combinations (TPR for each drug seed).

207

208 Furthermore, we used DComboNet to predict the combination potentially of all drug pairs. (**Fig.**

209 **3d**). In order to better analyze the prediction result, we further categorize drugs into 12 subtypes

210 based on their mechanism of action. We found drugs within the same subtype are more likely to be

211 recommended as combinable drugs because of their relevant functions, such as inhibitors of tyrosine

212 kinases and their receptors, drugs that interfere mitotic or target on microtubule (red box in **Fig. 3d**).

213 **Performance of level two model**

214 Performance of the DComboNet level two model was first evaluated using hepatocellular

215 carcinoma cell line HepG2 and breast adenocarcinoma cell line MCF7. Take HepG2 as an example,

216 gene expression profiles between HEPG2 and other cancer cell lines were compared to obtain

217 specifically expressed genes in HepG2; gene expression profiles of HepG2 before and after

218 monotherapy were compared to generate the differentially expressed genes (DEGs). HepG2 specific

219 network was constructed by added 632 HepG2 specifically expressed genes, 78913 drug-DEG, 1652

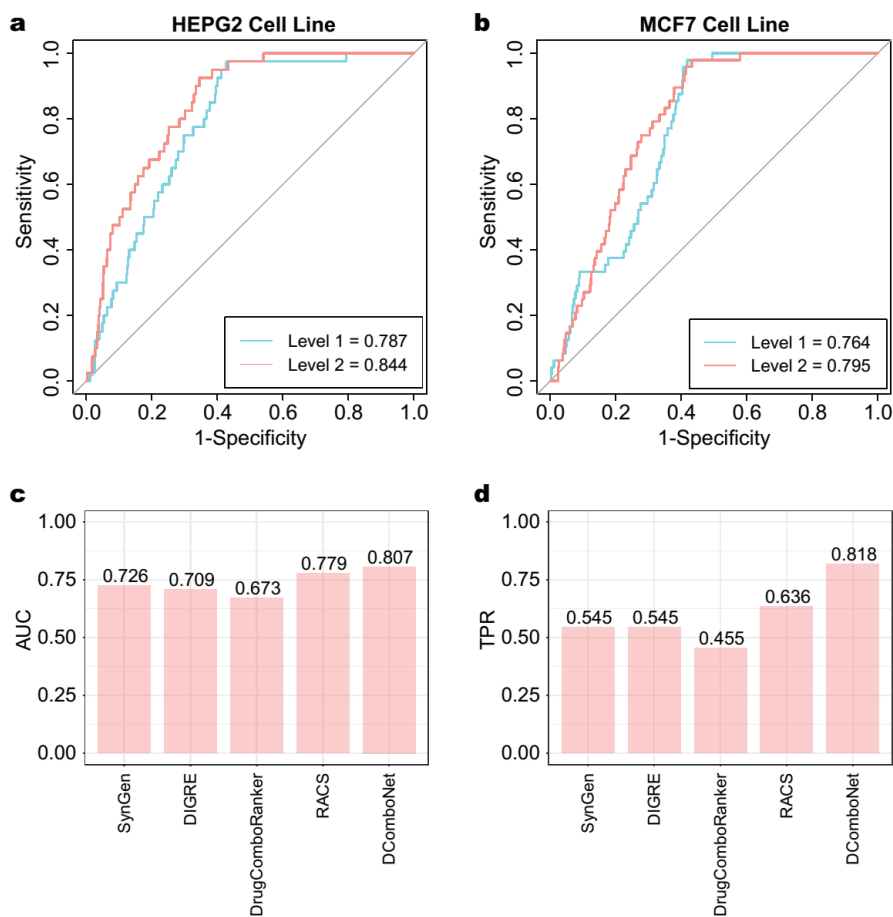
220 drug-DEpathway associations and the corresponding gene-gene, gene-drug and gene-pathway

221 associations. Potential drug combinations were predicted by level two model based on HepG2

222 specific network. The AUC of DComboNet level two model was 0.844 for HepG2, rising

223 significantly compared with AUC of level one model (AUC = 0.787, **Fig. 4a**). The prediction on

224 breast cancer cell lines MCF7 was also improved with the AUC rose from 0.764 to 0.795 (**Fig. 4b**).



225

226 **Figure 4.** Performance of cancer sample specific drug combination prediction model. **a)** and **b)** ROC curves of
227 model on hepatocellular carcinoma cell line, HEPG2, and breast cancer cell line, MCF7. In each plot, red and blue
228 curves indicate the ROC curves of level two model and level one model, and the number in legend indicate the AUC
229 values. **c-d)** Method comparison between DComboNet level two model and other prediction algorithms (SynGen,
230 DIGRE, DrugComboRanker, RACS) using the OCI-LY3 dataset.

231

232 Some drug combinations, especially standard chemotherapy that directly act on DNA/RNA,
233 cannot be correctly predicted in the level one model. By adding drug perturbed transcriptome
234 change, the effects of these drugs on cancer cells can be reflected through changes in a series of
235 genes or pathways instead of only their target genes, therefore correct prediction may be obtained.
236 For example, the combination of capecitabine and docetaxel are both standard chemotherapies with
237 a broad anti-cancer effect[21]. Although they show relatively high pharmacological drug similarity
238 ($sim_{DD}(\text{capecitabine}, \text{docetaxel}) = 0.695$), the target genes and the biological functions
239 between capecitabine and docetaxel are distinctive (target distance is 2, pathway similarity is 0.45)

240 [22, 23]. This combination failed at predicting as combinable pair in level one model, but was
241 successfully predicted in level two model after reconstructing cancer sample specific network.

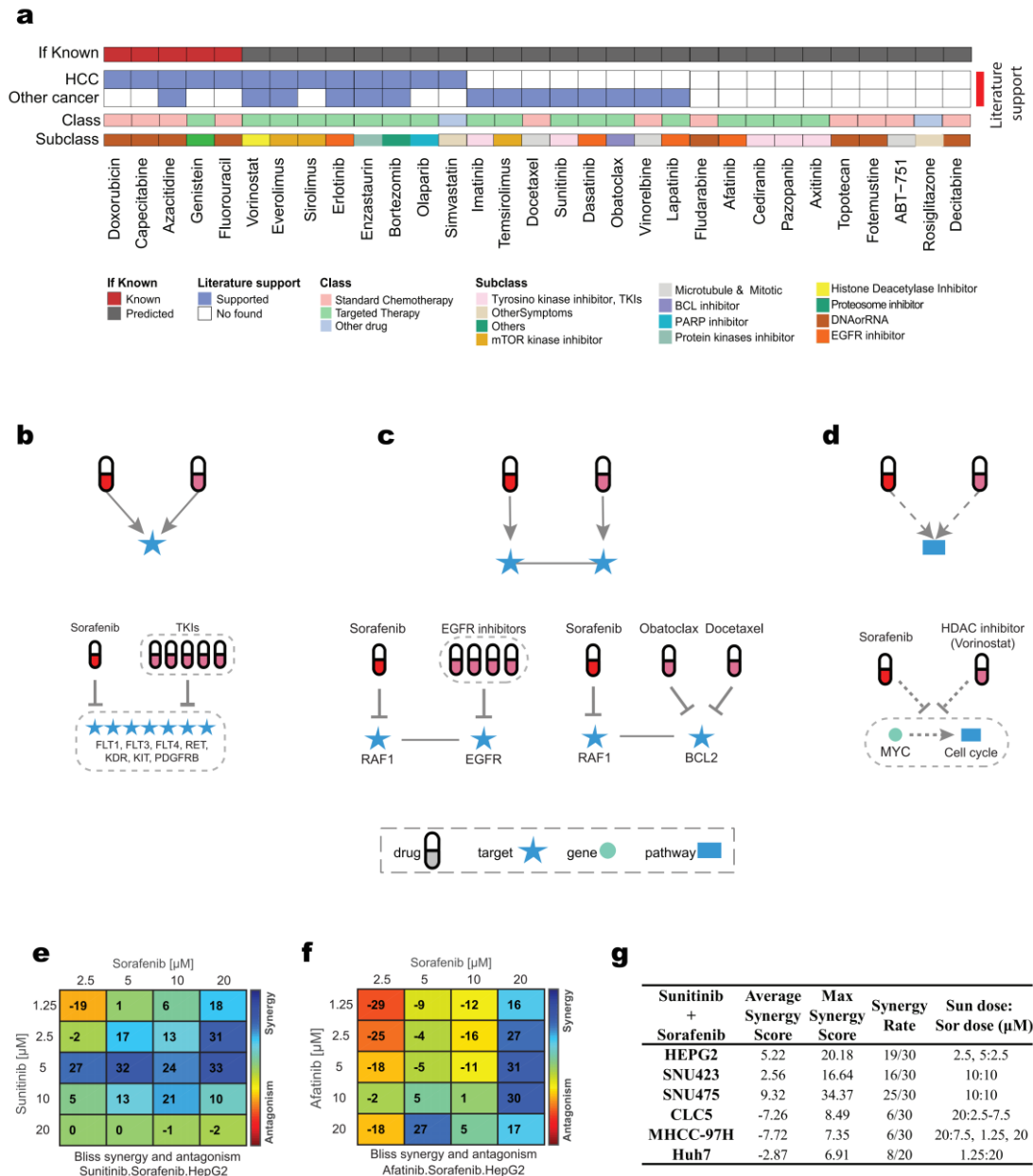
242 We further compared DComboNet level two model and other four drug combination prediction
243 algorithms (SynGen[9], DIGRE[10], DrugComboRanker [19], and RACS[11]) which also used the
244 change of transcriptome profiles before and after monotherapy treatments. All of these algorithms
245 were evaluated using the drug synergy screening dataset (OCI-LY3). The overall performance of
246 DComboNet outperformed other algorithms, especially more powerful when predicting the
247 combinable pairs (**Fig 4c-d**). DComboNet achieves 0.807 AUC and 81.8% true positive rate. Among
248 11 real synergy pairs, 9 pairs were successfully predicted by DComboNet while 7, 6, 6 and 5 pairs
249 were successfully predicted by RACS, SynGen, DIGRE and DrugComboRanker, respectively (**Fig**
250 **4d**).

251 **Case study: HepG2 - sorafenib**

252 Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death with only few
253 approved agents as first line treatment, such as sorafenib[24-26]. However, most patients will
254 develop sorafenib resistance eventually which include multiple biological pathways. Therefore, it
255 is critical to find potential drug combination to improve the efficacy of single sorafenib treatment.
256 We predicted combinable drugs for sorafenib treatment through DComboNet level two model and
257 validated the predictions through literature investigation and in-vitro experiments. Using ‘Sorafenib’
258 as the drug seed, 5 out of 6 known combinations were predicted correctly. In the rest 26 newly
259 predicted combinable drug pairs, 16 of them have been reported to be synergistic either in HCC
260 models (8 pairs) or in other cancers (8 pairs) in previous literatures (**Fig.5a**).

261 In addition to predicting the propensity of drug combinations, DComboNet can also rank genes
262 and pathways in the network according to the proximity relative to drug seed. Thus, analyzing the
263 overall results may be helpful in inferring the possible mechanism of drug combinations. Among
264 the drugs predicted as combinable candidates for sorafenib, we found three potential mechanism
265 modes for effective combination (**Fig 5b-d**).

266 The first mechanism is that two drugs shared same target genes (**Fig.5b**). Among the prediction
267 results, several multiple tyrosine kinase inhibitors (TKIs) show strong tendency to be combined
268 with sorafenib. Imatinib, cediranib, dasatinib, sunitinib and pazopanib shared 6 targets (FLT1, FLT3,
269 FLT4, KDR, KIT, PDGFRB) with sorafenib (Fig.5b). The combination between TKIs and sorafenib
270 may work on cancer-related genes or functions through compensatory way to avoid single TKI
271 resistance and further improve efficacy on cancer patients[27, 28]. For example, sorafenib can block
272 the function of genes related to imatinib resistance in HCC treatment [29].



273

274 **Figure 5.** Predictions and validation for Sorafenib on Hepatocellular carcinoma cell line, HEPG2 and the
 275 hypothetical mechanisms inferred. **a)** Overall prediction results for Sorafenib on HEPG2 cell line. The first line
 276 denotes if the predicted combinable drugs belong to known drug combination for Hepatocellular carcinoma (HCC);
 277 the second and third lines denote if predictions have literature supports for HCC or other cancer types; the fourth
 278 and fifth lines show the classification information of predicted drugs. **b-d)** indicate schematic diagrams of three
 279 potential combination mechanism modes for sorafenib case study. The upper part of **b)** shows the schematic diagram
 280 for drugs targeting on the same genes to achieve synergy, and the bottom shows the example "sorafenib-other
 281 tyrosine kinase inhibitors (TKIs)" matching this mode. The upper part of **c)** shows the mode that the interaction
 282 between drugs' target genes lead to synergy, and the bottom part shows two examples (sorafenib and EGFR inhibitor
 283 and sorafenib and BCL2 inhibitor). The upper part of **d)** shows the synergy may through the regulation of cancer-
 284 related genes other than target genes, and the matching examples (Sorafenib and HDAC inhibitors). In figure **b-d)**,
 285 the capsule shape nodes represent drugs; dark red corresponds to sorafenib, and light red corresponds to the predicted
 286 combinable drugs; blue stars represents target genes of drug; green round dots represents other genes in gene network;

287 and blue rectangles represent pathways. **e-f**) Experimental validation results for sorafenib combined with sunitinib
288 and afatinib, respectively. Each of the heatmaps shows the synergy score calculated by the Bliss method for each
289 dose points. The color bar of heatmap shows the score range from synergy (blue) to antagonism (red). **g**) The
290 summary table of experimental synergy screening for sorafenib and sunitinib in six hepatocellular carcinoma cell
291 lines (HEPG2, SNU475, SNU472, Huh-7, CLC5 and MHCC-97H) with multiple dose combinations.
292

293 The second mechanism is that two drugs may achieve synergy through the regulatory
294 relationships between their target genes (**Fig.5c**). Three epidermal growth factor receptor (EGFR)
295 inhibitors, erlotinib, afatinib and lapatinib[22, 30] were predicted as candidates with combination
296 potential with sorafenib. DComboNet showed that EGFR inhibitors connect to sorafenib through
297 the ‘EGFR-RAF1’ link (**Fig.5c**). EGFR is an upstream signal receptor Ras pathway while RAF1
298 acts as a signal transduction mediate with RAS/RAF/MEK/ERK signaling pathway [31, 32].
299 Inhibiting EGFR can help sensitize the efficacy of RAF inhibitor (e.g. sorafenib) in hepatocellular
300 cancer cell lines [33] and the synergism between RAF inhibitor sorafenib and EGFR inhibitors have
301 also been observed in multiple cancer types [34-36]. Another example is the predicted combination
302 of BCL2 inhibitor (docetaxel and obatoclox) and sorafenib. BCL2 inhibitor is connected with
303 sorafenib via the association of their target genes “BCL2-RAF1” in the network (**Fig.5c**). Over-
304 expression of BCL2 and RAF1 may lead to sorafenib resistance, which can be altered by inhibiting
305 BCL2 in HCC cell lines [37, 38]. This indicates that coadministration of BCL inhibitor and sorafenib
306 may improve treatment efficacy.

307 The third mechanism of drugs combination is that they co-regulate cancer-related genes, even
308 there are no direct target gene associations involved (**Fig.5d**). Take histone deacetylase (HDACs)
309 inhibitors vorinostat as an example (**Fig.5d**). Vorinostat itself plays an important anti-cancer role
310 which inhibit cancer cell growth via blocking cell cycle [39]. In the potential mechanism network,
311 we found sorafenib and vorinostat are linked together via down-regulating MYC (**Fig.5d**). HDACi

312 has been reported to help acetylate c-MYC and promote apoptosis in AML [40]. The sorafenib-
313 vorinostat combination may coregulate multiple pathways related to cancer cell cycle and apoptosis
314 to achieve synergism [41].

315 Based on these drug combination mechanisms, we selected two drugs (sunitinib and afatinib) to
316 further verify the predicted combination with sorafenib in HCC. Sunitinib shared 7 target genes with
317 sorafenib (**Fig.5b**), and their combination showed synergistic efficacy in renal cell carcinoma [42].
318 However, there are no similar studies in HCC. Therefore, we performed the combination
319 experiments of sorafenib and sunitinib using HCC cell line HepG2. 14 of the 20 dose combinations
320 showed synergy, and the most synergistic dose combination was when sunitinib was 5 μ M and
321 sorafenib was 20 μ M (**Fig.5e**). Furthermore, we verified a completely new prediction result, the
322 combination of sorafenib and afatinib. Afatinib is an EGFR inhibitor, which may achieve synergy
323 with sorafenib through the regulatory relationships between their target genes (**Fig.5c**). Experiments
324 in HepG2 showed 9 synergistic points at different dose, indicating that sorafenib and afatinib is
325 combinable (**Fig.5f**).

326 Additionally, the combination of sorafenib and sunitinib was further tested using another five
327 HCC cell lines (**Fig.5g**). SNU475 and SNU432 also showed synergy in experimental screening
328 especially strong synergy at multiple doses, while synergistic effect on Huh7, CLC5 and MHCC-
329 97H cell lines only occurred in few dose points. This reflects the heterogeneous response of cancer
330 cell lines to the same treatment. It is necessary to make individualized drug combination prediction.
331 If the expression profile of individual cancer sample is available, the DComboNet level two model
332 could obtain personalized prediction results by utilizing sample specific network.

333

334 **Discussion**

335 Discover efficient drug combination under the highly complex and heterogeneous cancer system
336 is difficult and time-consuming on wet-lab synergistic drug screening whereas in-silico drug
337 combination prediction has become a critical approach in preclinical research. Based on the
338 comprehension of anti-cancer drug mechanism and the accumulation of cancer related data, we
339 developed a two-level prediction model DComboNet. Level one model can be used to predict anti-
340 cancer drug combination in a more general manner, whereas level two model is capable to achieve
341 cancer sample specific drug combination prediction by integrating sample specific expressed genes,
342 differentially expressed genes and biological pathways after drug treatment into the ‘drug-
343 gene/pathway’ network.

344 DComboNet has several advantages: 1) DComboNet utilizes the complex multi-layer
345 heterogeneous networks, which efficiently integrate multi-level data and provide more information
346 to rank the combinable tendency from a holistic point of view. Therefore, DComboNet is possible
347 to predict drug pairs that have a more intricate combination mechanisms other than the direct target
348 gene association. 2) DComboNet contains two levels of models, which users can choose according
349 to their aim and available data. Level two model has better prediction accuracy than level one, but
350 requires the expression profiles of cancer sample before and after monotherapy treatment. 3)
351 Compared to other algorithms using similar input data, DComboNet achieves higher true positive
352 rate. 4) DComboNet provides drug-gene/pathway network between the predicted combinable drug
353 pairs, which is helpful for understanding the potential mechanism of drug synergy.

354 We noted that certain drug combinations are usually poorly predicted, especially those with lower

355 pharmacological similarities and less functionally relationship in gene or pathway network.
356 Additionally, DComboNet ranks candidate drugs based on their global similarity with the seed drug,
357 therefore it may have less power on predicting drug combinations with distinct mechanisms. With
358 the accumulation of high-throughput drug screening data, we plan to combine DComboNet with
359 supervised machine learning algorithms to improve the prediction performance. We also realized
360 that the different response between cancer cell lines and patients under the same drug treatment still
361 remains as a common obstacle for drug discovery transformation. Drug absorption, distribution,
362 metabolism and excretion process cannot be well modelled under the context of cancer cell lines.
363 Patient-derived mouse xenograft may serve as a better model than cell line for these, but drug
364 screening on animal model like mouse need more effort and funding. Although there are many
365 difficulties in the translation from basic research to application, computational prediction of drug
366 combinations is fast and convenient as well as achieves much better accuracy than random. We
367 anticipate that DComboNet could provide candidates for drug combination experiments and
368 accelerate the discovery of new synergistic drugs.

369 **Methods**

370 **Data collection**

371 Known drug combinations were collected from DCDB 2.0 [43]. Only FDA approved drugs or
372 drugs entering phase III or IV of clinical trial were kept in the subsequent analysis. Therapeutic
373 information used to construct drug-drug association network included: drug ATC (Anatomical
374 Therapeutic Chemical Classification System) codes[44] extracted from WHO Collaborating Centre
375 for Drug Statistics Methodology (WHOC) website, chemical structures downloaded from

376 DrugBank[22, 45] and PubChem[46], drug side effect information collected from SIDER4[47].

377 Drug target proteins or genes were retrieved from Drugbank and Therapeutic Target Database
378 (TTD)[48]; Drug related genes were obtained from IPA (Ingenuity® Pathway Analysis).

379 The human protein-protein interaction network were extracted from the scored InBio Map [49].

380 The interaction pairs with low score (score < 0.15) were removed. Cancer related genes were
381 extracted from KEGG Cancer related pathways [50].

382 Gene expression profiles of drug perturbed cancer cell lines were downloaded from LINCS
383 database[8] and DREAM challenge 2014[9]. LINCS database provided gene expression microarray
384 data for 1127 cell lines treated by 41847 molecules. Drug treated hepatocellular carcinoma cell line
385 HepG2 and breast cancer cell line MCF7 were extracted from LINCS. The pretreated gene
386 expression data of HepG2 and MCF7 were downloaded from CCLE database[6].

387 **Level one model: Cancer Drug Combination Network (DComboNet)**

388 Cancer Drug Combination Network (DComboNet) is based on a multi-level heterogenous
389 network which contains five subnetworks, drug-drug association network (N_{DD}), drug-gene
390 association network (N_{DG}) and gene-gene association network (N_{GG}), drug-pathway association
391 network (N_{DP}) and pathway-pathway association network (N_{PP}). The details of subnetwork
392 construction are described in Supplement methods. Briefly speaking, N_{DD} obtained drugs from
393 known drug combinations and their associations was weighted by pharmacological similarity
394 integrated three kinds of drug similarity. For level one model, N_{DG} was constructed based on drug
395 and target (D-T) associations, drug and drug-related gene (D-G) associations. N_{GG} integrated both
396 cancers related genes extracted from KEGG cancer related pathway including ‘pathway in cancer’

397 and genes connected with drugs in N_{DG} , and the associations between genes were extracted from
398 inBio Map (V 2016_09_12) [49]. N_{DP} was constructed based on the association between drugs
399 and their possible regulated pathways. N_{PP} was built on the hierarchy of KEGG provided in WNS
400 method [18].

401 The network can be represented as an adjacency matrix $A = \begin{bmatrix} A_{DD} & A_{DG} & A_{DP} \\ A_{GD} & A_{GG} & A_{PG} \\ A_{PD} & A_{GP} & A_{PP} \end{bmatrix}$, where A_{GD}

402 and A_{PD} are transpose of A_{DG} and A_{DP} . Given a certain drug, DComboNet recommends drugs
403 with closest topological relationship as the combinable candidates. This global proximity between
404 drugs can be captured via random walking with restart (RWR) algorithm. This algorithm originally
405 designed to simulate a random walker walking on the network with certain initial probability
406 corresponding network. For our task, we assigned the walk starts only from N_{DD} . More specific,

407 the random walker is assigned as a drug seed with an initial probability $P_0 = \begin{Bmatrix} [1 \ 0 \ \dots \ 0]_M^T \\ [0 \ \dots \ 0]_N^T \\ [0 \ \dots \ 0]_L^T \end{Bmatrix}$, where

408 M , N and L indicate the node number in N_{DD} , N_{GG} and N_{PP} , respectively. Walker will start
409 from this drug seed node to traverse every node in network. At every step, the jumping happens
410 from the current node to its direct neighbor(s) with a probability $1 - \sigma$ or returns to the drug node
411 with a restart probability σ . The probability in $t + 1$ step can be represented as follow:

$$P_{t+1} = (1 - \sigma)HP_t + \sigma P_0 \quad (1)$$

412 After several iterations, the probability will reach a steady state when the difference between P_{t+1}
413 and P_t falls below 10^{-10} . At this point, all nodes in the complex network have obtained global
414 proximity relative to the drug seed which can be considered as the combination potential. After
415 removing the known drug combinations, the rest candidate drugs can be ranked according to their
416 potential.

417 In function (1), $H = \begin{bmatrix} H_{DD} & H_{DG} & H_{DP} \\ H_{GD} & H_{GG} & O_{PG} \\ H_{PD} & O_{GP} & H_{PP} \end{bmatrix}$ denotes the transition matrix which reflects different

418 strategies for the walker to traverse the complex network.

419 The transition probability between drug 1 ($d1$) and drug 2 ($d2$) can then be described as:

420

$$H_{DD}(d1, d2) = \begin{cases} \frac{A_{DD}(d1, d2)}{\sum_{d=1}^M A_{DD}(d1, d)} & , \text{if } \sum_{g=1}^N E_{DG}(d1 \text{ or } d2, g) = 0 \text{ and } \sum_{p=1}^L E_{DP}(d1 \text{ or } d2, p) = 0 \\ \frac{\lambda_D A_{DD}(d1, d2)}{\sum_{d=1}^M A_{DD}(d1, d)} & , \text{others} \end{cases} \quad (2)$$

421 The jumping within drug network contains two different possibilities: if drug does not have any

422 link with N_{GG} or N_{PP} , the jump happens in N_{DD} with probability $\lambda_D = 1$; if $d1$ or $d2$ can be

423 linked to any gene node (g) and/or pathway node (p), the potential jumping happens within N_{DD}

424 with the probability λ_D .

425 If drug can be linked to N_{GG} , jumping from $d1$ to gene 1 ($g1$) may happen with the probability

426 λ_{DG} and the transition probability can be described as:

$$H_{DG}(d1, g1) = \begin{cases} \frac{\lambda_{DG} A_{DG}(d1, g1)}{\sum_{g=1}^N E_{DG}(d1, g)} & , \text{if } \sum_{g=1}^N E_{DG}(d1, g) \neq 0 \\ 0 & , \text{others} \end{cases} \quad (3)$$

427 After the jumping fall in N_{GG} , the transition probability from gene $g1$ to gene $g2$ in N_{GG} can

428 be influenced by the existence of edges in N_{GD} . Therefore, the transition probability within

429 N_{GG} can be computed as:

$$H_{GG}(g1, g2) = \begin{cases} \frac{A_{GG}(g1, g2)}{\sum_{g=1}^N A_{GG}(g1, g)} & , \text{if } \sum_{d=1}^M E_{DG}(g1 \text{ or } g2, d) = 0 \text{ and } \sum_{p=1}^L E_{GP}(g1 \text{ or } g2, p) = 0 \\ \frac{\lambda_G A_{GG}(g1, g2)}{\sum_{g=1}^N A_{GG}(g1, g)} & , \text{others} \end{cases} \quad (4)$$

430 Similarly, if jump happens from N_{GG} back to N_{DD} , transition probability between $g2$ and $d2$

431 can be described as:

$$H_{GD}(g2, d2) = \begin{cases} \frac{\lambda_{DG}A_{DG}(g2, d2)}{\sum_{d=1}^M E_{DG}(g2, d)} & , \text{if } \sum_{d=1}^M E_{DG}(g2, d) \neq 0 \\ 0 & , \text{others} \end{cases} \quad (5)$$

432 Similar to the jumping strategy through gene node, transition probability between $d1$ and
433 pathway node $p1$ can be calculated as:

$$H_{DP}(d1, p1) = \begin{cases} \frac{\lambda_{DP}A_{DP}(d1, p1)}{\sum_{p=1}^L E_{DP}(d1, p)} & , \text{if } \sum_{p=1}^L E_{DP}(d1, p) \neq 0 \\ 0 & , \text{others} \end{cases} \quad (6)$$

434 When the jump falls in N_{PP} , we expected the next step can directly happen from N_{PP} back to
435 N_{DD} . More specific, if the edge(s) between drug and pathway exist, jump can only happen either
436 within N_{PP} or between N_{DP} . The calculation of transition probability within N_{PP} can be seen as
437 follow:

$$H_{PP}(p1, p2) = \begin{cases} \frac{A_{PP}(p1, p2)}{\sum_{p=1}^L A_{PP}(p1, p)} & , \text{if } \sum_{d=1}^M E_{DP}(p1 \text{ or } p2, d) = 0 \\ \frac{\lambda_p A_{PP}(p1, p2)}{\sum_{p=1}^L A_{PP}(p1, p)} & , \text{others} \end{cases} \quad (7)$$

438 The jump from $p2$ back to $d2$ can then be described as:

$$H_{PD}(p2, d2) = \begin{cases} \frac{\lambda_{DP}A_{DP}(p2, d2)}{\sum_{d=1}^M E_{DP}(p2, d)} & , \text{if } \sum_{d=1}^M E_{DP}(p2, d) \neq 0 \\ 0 & , \text{others} \end{cases} \quad (8)$$

439 From the description of jumping strategy between $d1$ and $d2$, we can see that jumping
440 probability λ are not independent. Within the homological subnetworks, λ s are equal ($\lambda_D = \lambda_G =$
441 λ_P). The jumping probability in heterogenous subnetwork (such as N_{DG} and N_{DP}) are influenced
442 by those within homological subnetworks ($\lambda_{DG} = 1 - \lambda_D$ and $\lambda_{DP} = 1 - \lambda_D$).

443 To improve the accuracy of the model, global restart probability σ and jumping probabilities
444 (λ_D) were set to values from 0 to 1 and the optimal parameters were selected through cross-
445 validation (supplement figure 1). Based on the tuning results, the default setting of λ_D is set as 0.5
446 to keep the balanced contribution of sub-networks, and σ is set as 0.7 which is also consistence
447 with previous publications [51].

448 **Level two model: cancer-specific DComboNet**

449 To predict sample specific drug combination, transcriptome data before and after drug
450 perturbation were further integrated in the N_{DG} and N_{GG} as well as N_{DP} and N_{PP} to construct
451 sample specific complex network.

452 The specifically expressed genes (G_{cancer}) were selected with the criteria $\left| Expr_j - \frac{\sum_{j=1}^k Expr_j}{k} \right| >$
453 1.5 (that the fold change of gene expression between the specific cancer cell line and the average
454 of expression value of all the other cell lines above 1.5). G_{cancer} were used to replace the nodes in
455 N_{GG} for reconstruct cancer-specific gene-gene association network.

456 Cancer specific drug-gene and drug-pathway associations were added into the original drug-
457 gene/pathway association network N_{DG} and N_{DP} . These two associations were obtained by
458 comparing drug treated gene expression data and DMSO. Differentially expressed genes were
459 selected by functions `lmFit` and `eBayes` in the `Limma` package (FDR < 0.1) [52]. Differential
460 regulated pathways (DEpathway) were obtained by the GSEA algorithm (FDR < 0.1) [53]. The edge
461 weight of both drug-DEG were assigned as the fold changes of genes perturbed by drugs and drug-
462 DEpathway were assigned as 1. Furthermore, these differentially expressed genes and their protein-
463 protein interactions extracted from InBio Map were also added into N_{GG} .

464 Similar to Level two model, a given drug was set to be the seed with initial probability P_0 and
465 iterated till the steady status of the probability of nodes. Then the candidate drugs will be ranked
466 based on the final probability after removing the drug pairs in positive set.

467 **Cross validation and independent test**

468 We conducted Leave-One-Drug-pair-Out Cross Validation (LODOCV) to assess the model

469 performance. For each known drug combination, the edge weight was replaced by its integrated
470 pharmacological similarity score and every drug in the combination will be used as drug seed to
471 rank the rest of drugs in the network. Receiver operating characteristic (ROC) curves and the area
472 under these curves (AUC) were also used to quantify the performance. To access the successfully
473 predicted drug pairs and avoid the asymmetrical ranks, that is, the difference between the rank of B
474 when A is used as drug seed and the rank of A when B is taken as seed, a two-threshold strategy was
475 used to classify the drug pair into combinable, uncombinable and intermediate (Supplementary
476 method).

477 To further verify the predictability and generalization of our level two model in independent
478 dataset, we tested the model performance using the OCI-LY3 dataset [9]. Excess over Bliss (E.o.B.)
479 and signal to noise ratio (s.n.r.) were calculated by the Bliss independent model [9]. Drug pairs were
480 classified into combinable pairs (synergistic, E.o.B.>0 and s.n.r.>2) and uncombinable pairs
481 (antagonistic and additive).

482 **Experimental validation**

483 Potential combinations between sorafenib and sunitinib, sorafenib and afatinib were evaluated
484 using liver cancer cell lines (Supplementary method). Cell viability matrices of each drug pair on
485 the corresponding cell lines were used as the input data to calculate experimental synergy score with
486 Bliss model provided by Combenefit software [54]. To reflect the degree of synergy, the maximum
487 synergy score (Max Syn) and the synergy rate among all dose combinations was calculated.

488

489 **Code availability**

490 DComboNet is implemented in R language and available at
491 <https://github.com/VeronicaFung/DComboNet> .

492

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498 **AUTHOR CONTRIBUTIONS**

499 F.Y.M.F. and H.L. designed the study; F.Y.M.F. performed model construction and data analysis;
500 Z.T.Z. performed drug combination screening experiments; F.Y.M.F. wrote the manuscript; F.Y.M.F.
501 developed the R package; Y.X.L. and H.L. supervised research and revised the manuscript.

502 **DISCLOSURE DECLARATION**

503 The authors declare that they have no competing interests.

504

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