1   Crowdsourced identification of multi-target kinase inhibitors for RET- and TAU-

2   based disease: the Multi-Targeting Drug DREAM Challenge

3   **Short title:** Crowdsourced identification of multi-target kinase inhibitors

4   **Authors:** Zhaoping Xiong*, Minji Jeon*, Robert J Allaway*, Jaewoo Kang, Donghyeon

5   Park, Jinhyuk Lee, Hwisang Jeon, Miyoung Ko, Hualiang Jiang, Mingyue Zheng, Aik

6   Choon Tan, Xindi Guo, The Multi-Targeting Drug DREAM Challenge Community,

7   Kristen K Dang, Alex Tropsha, Chana Hecht, Tirtha K. Das, Heather A. Carlson, Ruben

8   Abagyan, Justin Guinney, Avner Schlessinger§, Ross Cagan§

9   *co-first authors

10 §corresponding authors

11

12 **Affiliations:**

13 RJA, JG, XG, NS, KKD: Sage Bionetworks, Seattle, WA

14 MJ, JK, DP, JL, MK: Department of Computer Science and Engineering, Korea

15 University, Seoul, Republic of Korea

16 JK, HJ: Interdisciplinary Graduate Program in Bioinformatics, Korea University, Seoul,

17 Republic of Korea

18 ACT: Department of Biostatistics and Bioinformatics, Moffitt Cancer Center, Tampa, FL,

19 USA

20 ZX: Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech

21 University, Shanghai 200031, China

22   HJ, MZ: Drug Discovery and Design Center, State Key Laboratory of Drug Research,

23   Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi

24   Road, Shanghai 201203, China

25   AT: Laboratory for Molecular Modeling, Division of Chemical Biology and Medicinal

26   Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel

27   Hill, NC, USA.

28   HAC: Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI, USA

29   RA: Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, San

30   Diego, CA, USA

31   AS, Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai,

32   New York, NY, USA. avner.schlessinger@mssm.edu

33   CH, TD, RC: Department of Cell, Developmental, and Regenerative Biology, Icahn

34   School of Medicine at Mount Sinai, New York, NY, USA

35   RC: Corresponding author. Contact information: Institute of Cancer Sciences, University

36   of Glasgow; Garscube Estate, Switchback Road, Bearsden, Glasgow Scotland G61

37   ross.cagan@glasgow.ac.uk

38

39

Abstract

40

41   A continuing challenge in modern medicine is the identification of safer and more

42   efficacious drugs. Precision therapeutics, which have one molecular target, have been

43   long promised to be safer and more effective than traditional therapies. This approach

44   has proven to be challenging for multiple reasons including lack of efficacy, rapidly

45   acquired drug resistance, and narrow patient eligibility criteria. An alternative approach

46   is the development of drugs that address the overall disease network by targeting

47   multiple biological targets ('polypharmacology'). Rational development of these

48   molecules will require improved methods for predicting single chemical structures that

49   target multiple drug targets. To address this need, we developed the Multi-Targeting

50   Drug DREAM Challenge, in which we challenged participants to predict single chemical

51   entities that target pro-targets but avoid anti-targets for two unrelated diseases: RET-

52   based tumors and a common form of inherited Tauopathy. Here, we report the results of

53   this DREAM Challenge and the development of two neural network-based machine

54   learning approaches that were applied to the challenge of rational polypharmacology.

55   Together, these platforms provide a potentially useful first step towards developing lead

56   therapeutic compounds that address disease complexity through rational

57   polypharmacology.

58

Author Summary

59

60   Many modern drugs are developed with the goal of modulating a single cellular pathway

61   or target. However, many drugs are, in fact, 'dirty;' they target multiple cellular pathways

62   or targets. This phenomenon is known as multi-targeting or polypharmacology. While

3

63 some strive to develop 'cleaner' therapeutics that eliminate secondary targets, recent

64 work has shown that multi-targeting therapeutics have key advantages for a variety of

65 diseases. However, while multi-targeting drugs that affect a precisely-defined profile of

66 targets may be more effective, it is difficult to computationally predict which molecules

67 have desirable target profiles. Here, we report the results of a competitive

68 crowdsourcing project (the Multi-Targeting Drug DREAM Challenge), where we

69 challenged participants to predict chemicals that have desired target profiles for cancer

70 and neurodegenerative disease.

71

Introduction

73　　Despite important advances in drug development, many diseases remain partly or

74 wholly resistant to drug-based treatments. In recent decades, the field has attempted to

75 address this by developing precision therapeutics with the goal of targeting critical

76 nodes in disease networks. However, this approach has proven to be challenging. Most

77 targeted therapeutics do not progress past preclinical research or clinical trials due to

78 poor efficacy or unacceptable toxicity [1,2]. Additional hurdles are encountered even

79 after clinical approval. For example, initial efficacy against melanoma by the BRAF

80 inhibitors dabrafenib or vemurafenib as single-agent therapeutics is generally followed

81 by emergent tumor resistance [3]. Further, based on biomarkers only a small number of

82 patients with metastatic tumors are eligible for these target-driven precision therapies,

83 and fewer still show sustained response [4].

84　　An alternative to developing drugs with single targets is the development of

85 polypharmacology (multi-targeting) drugs. Many clinically approved drugs bind multiple

4

86 targets (http://ruben.ucsd.edu/dnet) [5,6], and in some cases the drug is improved by

87 these secondary activities. For example, vandetanib, a drug used in RET-dependent

88 medullary thyroid cancer (MTC), has a broad range of kinase targets that include RET,

89 VEGFR, etc. [7]; pre-clinical studies suggest that some of these secondary targets can

90 contribute to drug efficacy (*e.g.*, [8,9]). Developing optimized polypharmacology-based

91 drugs remains a challenge, however, as there are few roadmaps for predicting

92 structures that combine beneficial targets ('pro-targets') while avoiding liabilities ('anti-

93 targets') in a single molecule.

94   Current approaches to predict multi-target compounds range from 'physics-based'

95 methods that directly predict binding affinities, to 'omics-based' approaches utilizing

96 large datasets such as those including side-effects or gene expression profiles [10–14].

97 Notably, recent advances in machine learning architectures have allowed the

98 development of more effective methods relying on both physics-based and omics-based

99 approaches [15,16]. For example, a generative tensorial reinforcement learning

100 (GENTRL) model was used to design a small-molecule with optimal biological activity

101 for the discoidin domain receptor 1 (DDR1) with desirable pharmacokinetic properties in

102 just 21 days [17]. However, several limitations remain: current methods often (i) identify

103 frequent binders or obvious compounds, (ii) fail to discriminate activities among close

104 analogs, or (iii) identify small molecule inhibitors of 'promiscuous kinases' (e.g., DDR1

105 [18]) that are inherently easier to target. Notably, exploration of the full chemical

106 space—estimated between 10^{24} and 10^{60} structures—still remains a tremendous

107 challenge: known binders of, *e.g.*, the kinome together address a limited set of chemical

108 scaffolds [19].

109   Rational polypharmacology will require an improved ability to predict chemical

110   structures that specifically target optimized target sets. To identify better computational

111   strategies for this problem, we developed the Multi-Targeting Drug DREAM Challenge

112   [20]. In this challenge, we challenged participants to predict single chemical entities that

113   target pro-targets but avoid anti-targets for two unrelated diseases: RET-based tumors

114   and an inherited form of Tauopathy. Exploring each disease, we used a Drosophila

115   'dominant genetic modifier screen' to identify mediators of Drosophila RET^{M955T}-

116   mediated transformation and of Tau-mediated dysfunction [21,22]. The resulting

117   functional suppressors and enhancers of RET transformation provide a 'roadmap' for

118   the ideal profile of a lead polypharmacological therapeutic.

119   These data provide therapeutic roadmaps of suppressors (candidate 'pro-targets')

120   and enhancers (candidate 'anti-targets') for RET- and TAU-based disease. We publicly

121   posted this data, challenging the community to develop computational approaches with

122   improved ability to predict single chemical structures that successfully inhibit pro-targets

123   while avoiding anti-targets. Here, we report the development of two artificial neural

124   network-based machine learning approaches that were applied to the challenge of

125   rational polypharmacology. Together, these platforms provide a potentially useful first

126   step towards developing lead therapeutic compounds that address disease complexity

127   through polypharmacology.

128

129

130

131 **Results**

132   Rational development of drugs that act through polypharmacology will require

133 identifying (i) pro-targets that, as a group, are predicted to be effective in addressing a

134 disease network, and (ii) anti-targets that are predicted to act as liabilities either due to

135 reduced disease efficacy or increased whole body toxicity. Our work in Drosophila [21–

136 27] identified whole body pro-targets and anti-targets for RET-associated cancer and

137 Tauopathy. To leverage this data, we established the Multi-targeting Drug DREAM

138 challenge (www.doi.org/10.7303/syn8404040) designed to promote and assess novel

139 rational approaches to polypharmacology (Figure 1).

140

141 *Figure 1 - Multi-targeting drug DREAM Challenge overview. This challenge sought*

142 *to identify new algorithms for the identification of multi-targeting drugs that bind disease-*

143 *relevant targets and avoid disease-specific anti-targets. Participating teams used a*

144 *broad array of different datasets and computational approaches to develop these*

145 *methods. They were then asked to use these methods to predict small molecules that*

146 *would bind to RET-driven cancer targets and anti-targets (Problem 1, P1) as well as*

147 *Tauopathy targets and ant-targets (Problem 2, P2). A shortlist of these predicted*

148 *molecules were then experimentally validated (Kd determination) as binders or non-*

149 *binders of P1 and P2 proteins. Finally, the submitted compounds were scored using a*

150 *combination of criteria including selectivity, novelty, patentability, and drug-like*

151 *properties to identify the top performing teams.*

152

153   *Pro-targets and anti-targets*

154   To ensure whole animal relevance, we used data from two Drosophila models. To

155   model RET-dependent oncogenic transformation, we used data from previous work that

156   screened Drosophila *GMR-RET^{M955T}* and *ptc>RET^{M955T}* cancer models [21,22]. Broad

157   genetic screening identified multiple key 'pro-targets' defined as 'dominant genetic

158   suppressors' of *GMR-RET^{M955T}*-mediated transformation including Drosophila orthologs

159   of BRAF, SRC, and S6K. Candidate 'anti-targets'—genetic loci that, when reduced,

160   acted as 'dominant genetic enhancers' of *RET^{M955T}*-mediated transformation—include

161   MKNK1, TTK, ERK8, PDK1, and PAK3. Given its central role, we also included RET as

162   a pro-target.

163   To provide a second independent test for compound predictions, we used data

164   recently generated in a screen of a previously-described inherited Drosophila

165   Tauopathy model [23–27]. Expressing the human disease isoform TAU^{R406W}

166   exogenously in a transgenic Drosophila line led to nervous system defects and animal

167   lethality [23–27]. Genetic screening of most of the Drosophila kinome identified several

168   dominant genetic suppressors of TAU^{R406W}. For example, reducing the activity of the

169   Drosophila orthologs of AURKA, PAK1, FGFR1, or STK11 suppressed TAU^{R406W}-

170   mediated fly lethality; these loci represent candidate therapeutic targets. Conversely,

171   reducing activity of PAK3, MAP3K7, or PIK3CA enhanced TAU^{R406W}-mediated lethality

172   (*unpublished data*), indicating they should be avoided by candidate therapeutics.

173   We used these two sets of data as benchmarks for computation-based compound

174   predictions: ideal therapeutic leads would inhibit most or all pro-targets while exhibiting

175   minimum activity against anti-targets.

8

176   *Participation and Performance*

177   The challenge had 190 registered participants. Of these registrants, 34 submitted

178   predictions, either as a part of one of 5 teams or as individuals. 10 prediction sets were

179   submitted for Problem 1, and 8 prediction sets were submitted for Problem 2. Each

180   prediction was scored using the rubric described in Table 1 (see Methods for additional

181   details).

Criteria	Points Awarded
Problem 1	
Binds RET[M918T)]	5
Binds BRAF, SRC, S6K	1, 3 or 9 if binding 1, 2 or 3 of these targets
Avoids MKNK1*	3
Avoids TTK, ERK8, PDK, PAK3	1, 2, 3, or 4 if it avoids 1, 2, 3, or 4 of these targets
Problem 2	
Binds AURKA*	5
Binds PAK1*	5
Binds FGFR1, LKB	1 or 3 if binding 1 or 2 of these targets
Avoids PAK3*	3
Avoids MAP3K7*	3
Avoids PIK3CA	1
Predicted to enter CNS	3
Both Problems	
Novelty	2
Ability to Patent	2
Drug-like molecule	3

182   **Table 1 - Scoring guidelines for Problems 1 and 2.** *Starred targets indicate that*

183   *binding or avoiding that target was a requirement to receive target-based points.*

184

185   *Problem 1: Prediction of multi-targeting compounds for RET-driven cancers*

9

186   Problem 1 asked participants to predict molecules that bound the RET^{M955T}-

187   associated cancer pro-targets RET[M918T], BRAF, SRC, S6K, and did not bind the

188   anti-targets MKNK1, TTK, ERK8, PDK1, and PAK3. Of the 10 prediction files submitted,

189   two of the predictions had the same top-ranked molecule and, therefore, 9 compounds

190   were selected by the challenge organizers for experimental evaluation. Supplemental

191   Figure 1A shows that while most of the submitted compounds had no similar molecule

192   submitted by another team (Tanimoto similarity >0.5), the few similar submitted

193   molecules were mostly predicted by the same method/team.

194   Binding evaluation revealed that the majority of predicted compounds (7/9) did not

195   bind any of the pro-targets at 10 μM or any of the anti-targets at 30 μM (Supplemental

196   Table 1). Two compounds were observed to significantly bind one or more pro-targets:

197   ZINC98209221 and ZINC40900273 (Figure 2, Table 2). ZINC98209221 was observed

198   to significantly bind all 4 pro-targets, while avoiding 2 of the 5 anti-targets. Of the

199   remaining 3 'bound' anti-targets, the K_d was larger than that of the pro-targets,

200   indicating that ZINC98209221 displayed a lower binding affinity for the Problem 1 anti-

201   targets as compared to the pro-targets. The other compound in Table 2,

202   ZINC40900273, only significantly bound one of 4 pro-targets (BRAF) but avoided all 5
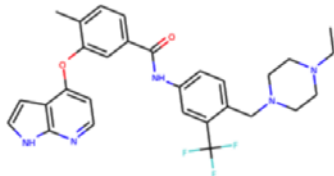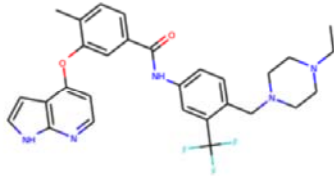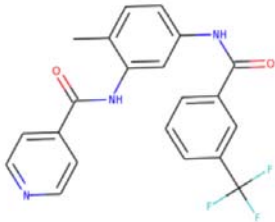
203   anti-targets.

Submitter or Team	Chemical ID	Structure	InChIKey
Zhaoping Xiong	ZINC98209221		SMPGEBOIKULBCT-UHFFFAOYSA-N
DMIS_MTD	ZINC98209221		SMPGEBOIKULBCT-UHFFFAOYSA-N
UM-BISBII	ZINC40900273		KUQAFPIBXZKZON-UHFFFAOYSA-N

204 **Table 2 - Compounds with activity against the Problem 1 targets.** *"Submitter or*

205 *Team" indicates the team that identified the compound as a solution to Problem 1.*

206 *"Chemical ID" is the ZINC identifier for the molecule. "Structure" is a two-dimensional*

207 *representation of the molecule. "InChIKey" is the hashed InChI for the molecule.*

208 *Strikingly, two teams identified the same molecule as a solution for Problem 1.*

209

210 **Figure 2 - Kd values of experimentally tested candidates from Problem 1.** *A*

211 *heatmap indicating the K_ds of all compounds tested in Problem 1. The best binder of the*

212 *pro-targets (ZINC98209221) was predicted by two different teams.*

213

214   *Problem 2: Prediction of multi-targeting compounds for Tauopathy*

215   Problem 2 asked participants to predict compounds that bind the Tauopathy pro-

216   targets AURKA, PAK1, FGFR1, and STK11 and avoid the Tauopathy anti-targets PAK3,

217   MAP3K7, and PIK3CA. Eight compounds were selected for experimental testing.

218   Kinase binding assays indicated that 6 of 8 of the predicted compounds did not bind any

219   of the pro-targets or anti-targets at 30 µM (Supplemental Table 2). Two compounds

220   were observed to bind one or more pro-targets: ZINC3938668 and ZINC538658 (Figure

221   3, Table 3). Supplemental Figure 1B recapitulates the finding from Problem 1 in

222   Problem 2 - most of the submitted compounds had no similar molecule submitted

223   (Tanimoto similarity >0.5) and when similar molecules were submitted, they were

224   generally predicted within a single method and not across different teams.
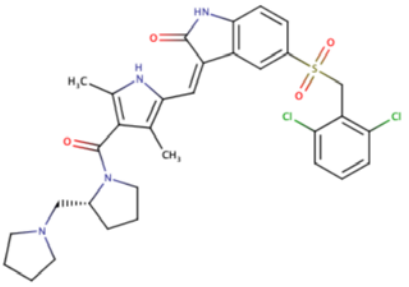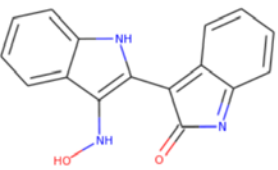
225

Submitter or Team	Chemical ID	Structure	InChIKey
Zhaoping Xiong	ZINC3938668		OYONTEXKYJZFHA-SSHUPFPWSA-N
DMIS_MTD	ZINC18279871		LDEWQRSYYHTQRA-UHFFFAOYSA-N

226

12

227 **Table 3 - Compounds with activity against the Problem 2 targets.** *"Submitter or*

228 *Team" indicates the team that identified the compound as a solution to Problem 2.*

229 *"Chemical ID" is the ZINC identifier for the molecule. "Structure" is a two-dimensional*

230 *representation of the molecule. "InChIKey" is the hashed InChI for the molecule.*

231

232 **Figure 3 - Kd values of experimentally tested candidates from Problem 2.** *A*

233 *heatmap indicating the K_ds of all compounds tested in Problem 1. ZINC3938668 and*

234 *ZINC538658 bind a subset of the pro-targets, and also both bind 2 of the three anti-*

235 *targets.*

236

237   The molecule predicted by Zhaoping Xiong, ZINC3938668, bound 3 of 4 pro-targets

238 at 10 µM: AURKA, PAK1, and STK11. However, this molecule also bound 2 of 3 anti-

239 targets at 10 µM: PAK3 and MAP3K7. Similarly, the predicted molecule from team

240 DMIS-MTD, ZINC538658, bound 3 of 4 pro-targets at 10 µM: AURKA, FGFR1, and

241 STK11, and 2 of 3 anti-targets at 10 µM: PAK3 and MAP3K7.

242 *Overall performance and methods summaries*

243   To identify the top participants, we used the scoring algorithm described in Table 1.

244 This algorithm considered multiple factors including binding of targets and avoiding anti-

245 targets as well as fulfilling novelty, patentability, and drug-likeness criteria to derive a

246 numerical score. The top teams for both Problem 1 and Problem 2 were identical:

247 Zhaoping Xiong and DMIS_MTD, with 16 points each for Problem 1, and 11 and 9

248 points for Problem 2, respectively (Table 4). The remaining teams received points for

249   novelty, patentability, and drug-likeness criteria, but the compounds predicted by these

250   teams failed to meet binding criteria to receive points. Therefore overall top-performers

251   for the challenge were Zhaoping Xiong and DMIS_MTD, with overall scores of 28 and

252   25, respectively (Table 4).

253

Submitter or Team	Problem 1	Problem 2	Interactome	Final Points
Zhaoping Xiong	16	11	TRUE	28
DMIS_MTD	16	9	FALSE	25
Lasige-BioISI - Multi-target Drug Designing	7	7	TRUE	15
SuperModels	7	7	FALSE	14
Huiyuan Chen	7	7	FALSE	14
Masahiro Mochizuki	7	7	FALSE	14
David Koes	7	7	FALSE	14
Gregory Koytiger	7	7	FALSE	14
UM-BISBII	8		FALSE	8
Stratified	7		FALSE	7

254   ***Table 4 - Challenge scoreboard.*** *"Submitter or Team" indicates which team was*

255   *scored. The "Problem 1" and "Problem 2" scores indicate each team's performance in*

256   *the two problems as scored using the criteria described in Table 1. "Interactome"*

257   *indicates whether the team participated in a text-mining exercise to facilitate team*

258   *interaction for bonus points. "Final Points" indicates the final score for each team.*

14

259   Across the 10 participating teams a variety of approaches were employed to arrive

260   at a set of predicted compounds for Problems 1 and 2 (Table 5 and Supplemental

261   Methods). The majority of these methods utilized multiple steps including (i) combining

262   structure- and ligand-based approaches, (ii) applying one or more machine

263   learning/classification algorithms, (iii) use of other computational approaches such as

264   filtering, ligand docking, or creation and application of confidence scoring metrics to

265   identify compounds for the two problems, or (iv) manual selection of the top candidates.

266   Importantly, some teams used fully automated methods, while others used approaches

267   that required several manual curation steps. While the majority of teams utilized one or

268   more machine learning approaches, the two top-performing teams specifically relied on

269   neural network-based approaches to identify the best compounds for Problem 1 and 2

270   (Table 2 & 3). We therefore examined these two approaches more closely to identify

271   key similarities and differences.

Team	Computational Approach Used	Manual Selection of Top Candidates	Method Data Description
David Koes	neural network	yes	PDB
Team Stratified	ensemble gradient boosting	yes	pIC50
DMIS-MTD	neural network	yes	Cmap scores
UM-BISBII	dataset filtering		SEA-TC algorithm
Lasige-BioISI - Multi-target Drug Designing	random forest, support vector machine	yes	ChEMBL
Gregory Koytiger	neural network		ChEMBL, SwissProt
SuperModels	random forest, logistic regression	yes	ChEMBL, Klaeger et al, Eidogen, ExCAPE-DB
Zhaoping Xiong	neural network	yes	Merget and Fulle et al, Uniprot
Masahiro Mochizuki	random forest, logistic regression		unspecified kinase inhibition assay dataset
Huiyuan Chen	similarity calculations		Drugbank

272 ***Table 5 - Methods summary table.*** *"Computational Approach Used" describes the*

273 *methods that were mentioned in each team's writeup (see Supplemental Methods).*

274 *"Manual Selection of Top Candidates" indicates whether a team described using a*

275 *manual curation step to produce their final set of predicted compounds. "Method Data*

276 *Description" highlights the data sources and data types used by each team.*

277

278 *Top performing teams*

279   The two teams that were most effective at predicting binding of pro-targets and

280 avoidance of anti-targets both used artificial neural network approaches. Method 1

281 (Zhaoping Xiong) used features derived from the protein and small molecules. Method 2

282 (Team DMIS_MTD) relied on transcriptional responses in combination with chemical

283 features of the drug, with a post processing step that included docking.

284   Method 1 used a graph convolutional neural network deep learning approach

285 [28,29] to predict candidate bioactive compounds (Figure 4). Ligand-based models are

286 constrained when testing is done with just a few bioactive compounds. To build a more

287 generalizable and useful model, this approach incorporated key features of each target

288 kinase by converting the amino acid sequence into a 'word embedding' vector, then

289 trained together with graph convolutional neural fingerprints. In developing this method,

290 different combinations were used of extended connectivity fingerprints (ECFP4) of

291 compounds with known bioactivity data, neural fingerprints, and protein embeddings.

292 Testing on a 10% internal hold-out test set demonstrated that the combination of neural

293 fingerprints and protein embeddings performed the best, with an RMSE of 0.414

294 (Supplemental Table 3).

295

296 **_Figure 4 -_** _A schematic summarizing Zhaoping Xiong's method. The molecule is_

297 _embedded with graph neural fingerprints and the target proteins are represented as_

298 _sequences and pretrained with a language model (doc2vec) model. These two_

299 _embeddings are fused together to classify the binding affinity as inactive, weak, or_

300 _potent._

301

302 The Method 1 researcher also made the observation that the majority of $-\log_{10} IC_{50}$

303 (pIC50) training data was centered around a pIC50 of 5, making prediction of more

304 extreme compound-target pIC50 values more challenging. To address this, the method

305 was developed as a classification problem in which the bioactivity of training data was

306 classified as "inactive" (pIC50 < 5), "weak" (5 <= pIC50 < 6) or "potent" (pIC50>=6). A

307 classification model trained on these data was then used to predict compound classes

308 for the targets/anti-targets posed by the Challenge. Finally, the top predictions were

309 manually selected based on predicted classes for the query targets and anti-targets.

310 Overall, this model performed best in the Challenge (Supplemental Figure 3).

311 Team DMIS-MTD based Method 2 on ReSimNet [30], a Siamese neural network

312 model that receives the structural information of two drugs and predicts their

313 transcriptional response similarity. ReSimNet was trained on the Connectivity Map

314 (CMap) dataset [31], which provides CMap scores of around 260,000 drug pairs from

315 2,400 unique drugs. The CMap score of a pair of drugs is computed based on the

316 similarity of the observed drug-induced gene expression profiles of the two drugs.

317   Trained on these CMap scores of observed drug pairs, ReSimNet predicts the CMap

318   scores of any drug pair that includes novel compounds.

319   Before utilizing ReSimNet to identify novel drug candidates, Team DMIS-MTD first

320   identified several prototype drugs with kinase inhibition profiles that satisfied most of the

321   Challenge's pro-target and anti-target conditions. They used the kinase inhibition

322   profiles from the *KI*nase *Ex*periment *O*mnibus (KIEO) database (http://kieo.tanlab.org),

323   which was constructed from experimental data on kinase inhibitors. The data was

324   curated from more than 600 published articles (Figure 5A). ReSimNet, trained on the

325   CMap dataset (Figure 5B), was used to predict the transcriptional response similarity

326   between a prototype drug and a novel compound from ZINC15 [32] (Figure 5C). Finally,

327   the top-ranking ZINC15 compounds were aggregated and filtered by Lipinski's rule, a

328   patent search through PubChem, and a protein-ligand docking algorithm (Figure 5D).

329

330   **Figure 5 -** *A schematic summarizing DMIS-MTD's method. The method was based on*

331   *ReSimNet [30], a Siamese neural network model that predicts their transcriptional*

332   *response similarity of two compounds. (A) Several prototype drugs whose kinase*

333   *inhibition profiles satisfy most of the pro-target and anti-target conditions of the*

334   *challenge problem were identified using the KInase Experiment Omnibus (KIEO)*

335   *database. (B-C) ReSimNet, trained on the CMap dataset , was used to predict the*

336   *transcriptional response similarity between a prototype drug and a novel compound*

337   *from ZINC15. (D) Finally, the top-ranking ZINC15 compounds are aggregated and*

338 *filtered for compounds that fulfill Lipinski's rules, a patent search through PubChem, and*

339 *a protein-ligand docking algorithm.*

340

341 *Translation of hits to in vivo model systems*

342 After identifying the best-binders in the competitive phase of the Challenge, we

343 assessed the *in vivo* translatability of the *in silico* predicted binders from Problem 1 that

344 were also found to bind the P1 targets *in vitro* (Table 2, Figure 2A). We obtained and

345 tested the compounds for their ability to rescue viability of transgenic flies that

346 expressed the oncogenic form of Drosophila RET in multiple tissues (*ptc>RET^{M955T}*) as

347 previously described [22,33].

348 Control transgenic *ptc>RET^{M955T}* (RET2B) flies fail to eclose to adulthood due to

349 expression of the transgene (0% adult viability; Supplemental Figure 4, Supplemental

350 Table 4). Feeding *ptc>RET^{M955T}* flies with food containing AD80 or APS6-45 (50 µM)—

351 experimental multi-targeting kinase inhibitors that reduce *ptc>RET^{M955T}* lethality

352 [22,33]—significantly increased survival to 27% and 22% mean eclosure, respectively

353 (Supplemental Figure 4, Supplemental Table 4). Feeding with ZINC98209221 or

354 ZINC40900273 (1, 10, or 50 µM final food concentrations) rescued to 1-1.5% of

355 animals, respectively (Supplemental Figure 4, Supplemental Table 4), a number that

356 failed to rise to statistical significance.

357 *Ensemble modeling*

358 As a proof-of-concept to identify candidates for future study, we devised a

359 "wisdom of the crowds" approach to identify consensus Problem 1 predictions of the

19

360   top-performing models by identifying the nexus of chemical space predicted by these

361   methods. Specifically, we obtained a larger list of top predictions from the two top-

362   performing teams using the same method and then determined the pairwise Tanimoto

363   similarity (Supplemental Figure 2A) between these two sets. Using this data, we

364   performed network analysis (Supplemental Figure 2B), which allowed us to identify

365   subclusters of structurally similar predicted molecules within the full set of top predicted

366   molecules. We further profiled these molecules using computationally generated

367   absorption, distribution, metabolism and excretion (ADME) metrics (Supplemental Table

368   5) to facilitate future prioritization of these molecules for medicinal chemistry and *in vivo*

369   screening.

370

Discussion

One approach to overcoming the limitations of precision medicine approaches in drug development is to develop new drugs with a goal of multi-targeting drugs that target multiple nodes in a disease network. Developing these drugs is a challenge, as both desired targets as well as toxic targets must be considered in the design process. One possible framework for identifying these drugs involves the use of predictive computational modeling strategies to perform virtual screening and identify compounds with a desirable target profile. Here, we described our efforts to identify better computational approaches for this pharmacologic problem.

To provide a disease context to this challenge, we used a set of previously identified pro-targets and anti-targets to design two challenge problems. Problem 1 asked teams to predict molecules that bound the RET^{M955T}-associated cancer targets RET[M918T], BRAF, SRC, S6K, and avoided binding to MKNK1, TTK, ERK8, PDK1, and PAK3. Problem 2 asked participants to predict compounds that bound the Tauopathy pro-targets, AURKA, PAK1, FGFR1, and STK11 and did not bind PAK3, MAP3K7, and PIK3CA. The teams collectively identified 3 compounds for Problem 1 and 2 compounds for Problem 2 that were observed to have any binding potency to the pro-targets. Furthermore, the same set of teams in both problems were successful, indicating that their methods were consistently best-performing. Both of the top-performing teams used neural networks based learning approaches. However, there were other teams that also employed a neural network based approach but did not successfully identify any binding compounds, suggesting that the specific approach may be critical for success. Other approaches (solely manual curation, random forest,

21

394 logistic regression) were unsuccessful in identifying binders, suggesting that these

395 methods may not be well-poised to perform well in this type of problem.

396 Another key finding was that most of the submitted methods, including the top-

397 performing methods, required a manual curation/selection step to select the top

398 performing compounds (Table 5). The decisions and criteria that are used in manual

399 selection can be difficult to define or reproduce using a decision tree or other algorithm.

400 A clear direction for future studies is to better define the nature and rationale for these

401 manual curation steps and devise strategies to automate these steps. The benefits of

402 such future work would likely be two-fold: it would increase the reproducibility of these

403 methods, and it could yield ensemble methods that are more successful in identifying

404 optimal compounds for a given set of targets and anti-targets.

405 The predominant limitation of this challenge was a relatively small participant pool,

406 which likely resulted in constrained space of methodology. Remarkably, the top-

407 performing methods predicted the identical compound (ZINC98209221) as the best

408 candidate for solving Problem 1, which is surprising given the size of the ZINC15

409 compound library. Furthermore, previous DREAM Challenges have consistently

410 demonstrated that a variety of methodologic approaches can perform well in many

411 different applications; therefore, it may be the case that methodologic approaches

412 beyond neural networks may indeed be applicable to the problems defined in this

413 challenge, but that the implementation may need to be altered to be successful.

414 The other key limitation of this study was the difficulty in translating computationally

415 predicted best binders to activity in an *in vivo* system. We tested the top prediction from

416 Problem 1 in the Drosophila RET^{M955} model system of RET-driven cancer but did not

22

417   observe bioactivity. There are multiple possible explanations for this, such as the

418   standard ADME challenges faced in drug development, as well as the differences

419   between the fly and human targets and networks [22,34]. To ameliorate this,

420   improvements could include increasing the scale of experimentally tested compounds to

421   identify a larger number of best binders, performing more stringent computational

422   filtering to eliminate compounds with poorer predicted bioavailability characteristics, and

423   performing standard medicinal chemistry and structure-activity relationship studies to

424   identify analogues with better *in vivo* performance.

425   In the Results, we described a proof-of-concept approach to use these methods to

426   identify molecular candidates for future experimental validation. By leveraging a

427   "wisdom of the crowds" approach, we identified consensus Problem 1 predictions of the

428   top-performing models by identifying the nexus of chemical space predicted by these

429   methods. We subsequently evaluated these molecules using computationally ADME

430   metrics to facilitate future prioritization of these molecules for medicinal chemistry and *in*

431   *vivo* screening. With this in mind, employing a tandem approach where that leverages

432   ensembled computational models to identify a general region of chemical space with an

433   appropriate polypharmacological profile, followed by more traditional drug optimization

434   approaches, may be a worthwhile future avenue to accelerate the development of multi-

435   targeting drugs.

Materials and Methods

Challenge infrastructure

Multi-targeting Drug DREAM Challenge participants were required to submit a prediction file following a pre-formatted YAML template that included each team's top 5 compound predictions for each of the two problems (RET^{M955T1}- and TAU^{R406W}-mediated phenotypes) (Figure 1). Each prediction included a compound name, vendor name and ID, a methods description, a rationale explaining why the approach was innovative, how the approach is generalizable, and why top predicted compounds are chemically novel. Participants were restricted to compounds that were available through a vendor listed on ZINC15 or other vendors for no more than $250 per mg. The full set of predictions and methods descriptions provided by the Multi-targeting Drug DREAM Challenge community is available as Supplementary Methods.

In vitro testing

Compounds were purchased and delivered to a contract research organization for experimental testing of binding to the target and anti-target kinases defined for Problems 1 (Figure 2) and 2 (Figure 3). Binding was evaluated using the DiscoverX KinomeScan profiling service. In brief, this method evaluated the ability of the predicted compounds to compete with a bead-immobilized ligand for the active sites of DNA-tagged kinases from Problem 1 and Problem 2. Kinases bound to the immobilized ligand were exposed to a broad range of concentrations of the prediction compounds (11 doses, 3-fold serial dilutions starting at 30 µM of compound). At each dose, the amount of drug bound to kinase was measured by eluting the non-bead-bound DNA-

24

458 tagged kinase and performing quantification by quantitative polymerase chain reaction

459 (qPCR).

460 *Scoring Algorithm*

461 Submissions were assessed using a point-based approach (Table 1). Points were

462 awarded for meeting multiple criteria, in which "binding" of a pro-target was a positive hit

463 at 10 µM and "non-binding" of an anti-target was defined as a negative result at 30 µM

464 (Supplemental Tables 1,2).

465 For problem 1, each of the compounds submitted were awarded points for binding

466 RET[M918T], BRAF, SRC, and/or S6K. Compounds were also awarded points for

467 avoiding MKNK1, TTK, ERK8, PDK1, and/or PAK3. For problem 2: E, each of the

468 compounds submitted were awarded points for binding AURKA, FGFR1, LKB1, and

469 PAK1 and avoiding the anti-targets PAK3, MAP3K7 (TAK1), and PIK3CA. In addition,

470 points were awarded for predictions for meeting other criteria including: novelty

471 (ECFP6-based Tanimoto coefficient <0.4 against all other ChEMBL compounds known

472 to be active against these targets), ability to patent (<2 hits in SciFinder database), and

473 drug-like features (adheres to ¾ of Lipinski's rule of 5). In addition, for Problem 2,

474 predictions predicted to enter the central nervous system (polar surface area <75) were

475 awarded additional points.

476

477 *Chemical similarity calculations*

478 Chemical similarity calculations and visualizations were performed in the R

479 programming language using rcdk, fingerprint, pheatmap and visNetwork packages.

25

480   SMILES strings for each structure were provided by each team, and then converted to a

481   binary fingerprint using the "standard" method provided by the fingerprint package (a

482   1024-bit "path based, hashed fingerprint"). These fingerprints were then used to

483   generate pairwise similarity matrices using the fingerprint::fp.sim.matrix with the method

484   parameter set to 'tanimoto'. Full R notebooks describing these analyses can be found at

485   https://github.com/Sage-Bionetworks-Challenges/Multi-Target-Challenge.

486

487   *Ensemble method*

488   Ensemble predictions were performed by generating lists of top predictions for each

489   top-performing team and assessing structural similarity as described above. We then

490   used the SwissADME [35] webapp to calculate the number of Lipinski violations, Ghose

491   violations, Veber violations, Egan violations, Muegge violations, PAINS alerts, Brenk

492   alerts, and Lead-likeness violations (Supplemental Table 5). For each compound and

493   we assigned a score of 1 for each value without a violation or alert, or a score of 0

494   otherwise. We then summed these values to create a composite score where a larger

495   score indicates fewer applicable violations/alerts to prioritize compounds for in vivo

496   screening.

497

498   *In vivo validation*

499   Female *UAS-RET^{M955T}* virgins were mated to *ptc-GAL4* males to generate *ptc>RET^{M955T}*

500   progeny. Progeny were grown at 25°C and compounds were tested by feeding larvae at

501   three doses: 10 µM, 5 µM, and 1 µM final food concentration. DMSO alone served as a

502   negative control and the experimental compounds AD80 and APS6-45 [22,33] served

503   as positive controls. Using survival to adulthood (eclosure), each condition was done in

504   quadruplicate, with 41-110 animals tested for each drug concentration.

505

506   *Code availability*

507   Zhaoping Xiong's top-performing method is available in a Github repository:

508   https://github.com/xiongzhp/FusedEmbedding. Instructions for running the method can

509   be found here:

510   https://github.com/xiongzhp/FusedEmbedding/blob/master/REPRODUCING.md.

511   Team DMIS-MTD's top-performing method is available at:

512   https://github.com/dmis-lab/ReSimNet

513   https://www.synapse.org/#!Synapse:syn20545440

514   Instructions for running this method can be found at:

515   https://github.com/dmis-lab/ReSimNet/blob/master/README.md.

516   A detailed description of the implementation and results of these two methods as well

517   as all other submitted methods can be found in the Supplemental Methods. Post

518   challenge analysis notebooks can be retrieved from:

519   https://github.com/Sage-Bionetworks-Challenges/Multi-Target-Challenge.

520

521   **Acknowledgements**

536

Contributions

538   RJA, JG, XG, NS, MJ, AT, HAC, RA, AS, ZX, HJ, MZ, AS, and RC wrote and edited the

539   manuscript. JG, XG, NS, KD, MJ, KJ, DP, JL, HJ, MK, ACT, AT, HAC, RA, AS, and RC

540   designed and implemented or competed in the competitive phase of the challenge. RJA,

541   XG, KD, RA, CH, TK, AS, and RC designed and implemented post-challenge

542   experiments and analyses. JG, KD, AS, RC were responsible for procuring funding for

543   this study and supervising the work.

544

28

545 **Conflict of interest statement**

546 Avner Schlessinger is co-founder of AIchemy, LLC. RC is a consultant to My Personal
547 Therapeutics/ Vivan.
548

References

1. Barton P, Riley RJ. A new paradigm for navigating compound property related drug attrition. Drug Discovery Today. 2016. pp. 72–81. doi:10.1016/j.drudis.2015.09.010

2. Empfield JR, Clark MP. Reducing Drug Attrition. Springer; 2014.

3. Sullivan RJ, Flaherty KT. Resistance to BRAF-targeted therapy in melanoma. Eur J Cancer. 2013;49: 1297–1304.

4. Marquart J, Chen EY, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Benefit From Genome-Driven Oncology. JAMA Oncol. 2018;4: 1093–1098.

5. Allaway RJ, La Rosa S, Guinney J, Gosline SJC. Probing the chemical–biological relationship space with the Drug Target Explorer. Journal of Cheminformatics. 2018. doi:10.1186/s13321-018-0297-4

6. Shi D, Khan F, Abagyan R. Extended Multitarget Pharmacology of Anticancer Drugs. J Chem Inf Model. 2019;59: 3006–3017.

7. Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, et al. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol. 2011;29: 1046–1051.

8. Vidal M, Wells S, Ryan A, Cagan R. ZD6474 suppresses oncogenic RET isoforms in a Drosophila model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. Cancer Res. 2005;65: 3538–3541.

9. Vitagliano D, De Falco V, Tamburrino A, Coluzzi S, Troncone G, Chiappetta G, et al. The tyrosine kinase inhibitor ZD6474 blocks proliferation of RET mutant medullary thyroid carcinoma cells. Endocr Relat Cancer. 2011;18: 1–11.

10. Szalai B, Subramanian V, Holland CH, Alföldi R, Puskás LG, Saez-Rodriguez J. Signatures of cell death and proliferation in perturbation transcriptomics data-from confounding factor to effective prediction. Nucleic Acids Res. 2019;47: 10010–10026.

11. Cichonska A, Ravikumar B, Allaway RJ, Park S, Wan F, Isayev O, et al. Crowdsourced mapping of unexplored target space of kinase inhibitors. bioRxiv. 2020. p. 2019.12.31.891812. doi:10.1101/2019.12.31.891812

12. Thafar M, Raies AB, Albaradei S, Essack M, Bajic VB. Comparison Study of Computational Prediction Tools for Drug-Target Binding Affinities. Front Chem. 2019;7: 782.

13. Pabon NA, Xia Y, Estabrooks SK, Ye Z, Herbrand AK, Süß E, et al. Predicting

583   protein targets for drug-like compounds using transcriptomics. PLoS Comput Biol.
584   2018;14: e1006651.

585 14. Douglass EF, Allaway RJ, Szalai B, Wang W, Tian T, Fernández-Torras A, et al. A
586   Community Challenge for Pancancer Drug Mechanism of Action Inference from
587   Perturbational Profile Data. bioRxiv. 2020. p. 2020.12.21.423514.
588   doi:10.1101/2020.12.21.423514

589 15. Lim H, Xie L. Omics Data Integration and Analysis for Systems Pharmacology.
590   Methods Mol Biol. 2019;1939: 199–214.

591 16. Schneider P, Walters WP, Plowright AT, Sieroka N, Listgarten J, Goodnow RA Jr,
592   et al. Rethinking drug design in the artificial intelligence era. Nat Rev Drug Discov.
593   2020;19: 353–364.

594 17. Zhavoronkov A, Ivanenkov YA, Aliper A, Veselov MS, Aladinskiy VA, Aladinskaya
595   AV, et al. Deep learning enables rapid identification of potent DDR1 kinase
596   inhibitors. Nat Biotechnol. 2019;37: 1038–1040.

597 18. Hanson SM, Georghiou G, Thakur MK, Miller WT, Rest JS, Chodera JD, et al.
598   What Makes a Kinase Promiscuous for Inhibitors? Cell Chem Biol. 2019;26: 390–
599   399.e5.

600 19. Rahman R, Ung PM-U, Schlessinger A. KinaMetrix: a web resource to investigate
601   kinase conformations and inhibitor space. Nucleic Acids Res. 2019;47: D361–
602   D366.

603 20. Schlessinger A, Abagyan R, Carlson HA, Dang KK, Guinney J, Cagan RL. Multi-
604   targeting Drug Community Challenge. Cell Chem Biol. 2017;24: 1434–1435.

605 21. Read RD, Goodfellow PJ, Mardis ER, Novak N, Armstrong JR, Cagan RL. A
606   Drosophila model of multiple endocrine neoplasia type 2. Genetics. 2005;171:
607   1057–1081.

608 22. Sonoshita M, Scopton AP, Ung PMU, Murray MA, Silber L, Maldonado AY, et al. A
609   whole-animal platform to advance a clinical kinase inhibitor into new disease space.
610   Nat Chem Biol. 2018. doi:10.1038/nchembio.2556

611 23. Beharry C, Alaniz ME, Alonso ADC. Expression of Alzheimer-like pathological
612   human tau induces a behavioral motor and olfactory learning deficit in Drosophila
613   melanogaster. J Alzheimers Dis. 2013;37: 539–550.

614 24. Blard O, Feuillette S, Bou J, Chaumette B, Frébourg T, Campion D, et al.
615   Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in
616   Drosophila. Hum Mol Genet. 2007;16: 555–566.

617 25. Grammenoudi S, Anezaki M, Kosmidis S, Skoulakis EMC. Modelling cell and
618   isoform type specificity of tauopathies in Drosophila. SEB Exp Biol Ser. 2008;60:

619   39–56.

620   26. Pfleger CM, Wang J, Friedman L, Vittorino R, Conley LM, Ho L, et al. Grape-seed
621   polyphenolic extract improves the eye phenotype in a Drosophila model of
622   tauopathy. Int J Alzheimers Dis. 2010;2010. doi:10.4061/2010/576357

623   27. Williams DW, Tyrer M, Shepherd D. Tau and tau reporters disrupt central
624   projections of sensory neurons in Drosophila. J Comp Neurol. 2000;428: 630–640.

625   28. Li X, Li Z, Wu X, Xiong Z, Yang T, Fu Z, et al. Deep Learning Enhancing Kinome-
626   Wide Polypharmacology Profiling: Model Construction and Experiment Validation. J
627   Med Chem. 2019. doi:10.1021/acs.jmedchem.9b00855

628   29. Xiong Z, Wang D, Liu X, Zhong F, Wan X, Li X, et al. Pushing the Boundaries of
629   Molecular Representation for Drug Discovery with the Graph Attention Mechanism.
630   J Med Chem. 2019. doi:10.1021/acs.jmedchem.9b00959

631   30. Jeon M, Park D, Lee J, Jeon H, Ko M, Kim S, et al. ReSimNet: drug response
632   similarity prediction using Siamese neural networks. Bioinformatics. 2019;35:
633   5249–5256.

634   31. Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, et al. A Next
635   Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. Cell.
636   2017;171: 1437–1452.e17.

637   32. Sterling T, Irwin JJ. ZINC 15--Ligand Discovery for Everyone. J Chem Inf Model.
638   2015;55: 2324–2337.

639   33. Dar AC, Das TK, Shokat KM, Cagan RL. Chemical genetic discovery of targets and
640   anti-targets for cancer polypharmacology. Nature. 2012;486: 80–84.

641   34. Ung PMU, Sonoshita M, Scopton AP, Dar AC, Cagan RL, Schlessinger A.
642   Integrated computational and Drosophila cancer model platform captures
643   previously unappreciated chemicals perturbing a kinase network. PLoS Comput
644   Biol. 2019;15: e1006878.

645   35. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate
646   pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small
647   molecules. Sci Rep. 2017;7: 42717.

648

649

650 **Supplemental Figure Legends**

651 **Supplemental Figure 1 -** Chemical similarity comparison of all submitted Problem 1

652 and Problem 2 solutions.

653 **Supplemental Figure 2 -** (A) Similarity heatmap of top predicted compounds for

654 Problem 1 provided by the top-performing teams. Columns correspond to predictions

655 from Zhaoping Xiong, while rows correspond to predictions from DMIS-MTD. The

656 majority of compounds are relatively dissimilar from one another. (B) The similarity

657 matrix was converted into a network, where nodes are individual predicted compounds

658 and edges encode compound-compound Tanimoto similarities. Edge thickness

659 represents similarity (thicker edges = greater similarity). Edges representing similarity

660 below 0.4 were filtered out, and cluster subnetworks were identified (with each color

661 representing an individual subnetwork).

662 **Supplemental Figure 3 -** Recapitulating Zhaoping Xiong's three-class classification

663 model used for screening. (A) The distribution of pIC50; (B) The distribution of classes;

664 (C) The accuracy of the model across train, valid and test sets

665 **Supplemental Figure 4 -** RET2B and control *D. melanogaster* were treated with DMSO

666 (negative control), two candidate RET-targeting compounds identified using top

667 performing methods (ZINC4020 and ZINC9820) and two positive control multi-targeting

668 RET inhibitors previously demonstrated to rescue the RET2B model (AD80 [33], APS6-

669 45 [22]). Bars show the mean percent survival and error bars show the standard

670 deviation of four replicates per condition. A Mann-Whitney test was used to assess the

671 significance of changes to percent survival in the varying conditions. The RET2B Adult

672 panel (bottom right) indicates that higher concentrations of AD80 or APS6-45

33

673   significantly ($p < 0.05$) rescue the RET2B model, while the two candidate ZINC molecules

674   have a minimal and non statistically-significant effect on percent survival to adulthood.

675

676

677 **Supplemental Table Legends**

678 **Supplemental Table 1 - Problem 1 Results.** "Submitter or Team" indicates which team

679 was scored. "Chemical ID" indicates the ZINC or MolPort identifier for each predicted

680 compound. "InChIKey" indicates the InChiKey for each predicted compound. Columns

681 4-12 indicate the experimentally-validated binding constant (K_d, nanomolar) for the pro-

682 target or anti-target listed in the column header (>30000 nM indicates it was above the

683 detection limit for the assay). Columns 13-16 indicate the status of the compound with

684 respect to the challenge rules for novelty, drug-like properties, and likelihood to pass the

685 CNS as defined in the challenge scoring rules. The final column indicates the number

686 of points the prediction was given based on the defined scoring criteria.

687 **Supplemental Table 2 - Problem 2 Results.** "Submitter or Team" indicates which team

688 was scored. "Chemical ID" indicates the ZINC or MolPort identifier for each predicted

689 compound. "InChIKey" indicates the InChiKey for each predicted compound. Columns

690 4-10 indicate the experimentally-validated binding constant (K_d, nanomolar) for the pro-

691 target or anti-target listed in the column header (>30000 nM indicates it was above the

692 detection limit for the assay). Columns 11-14 indicate the status of the compound with

693 respect to the challenge rules for novelty, drug-like properties, and likelihood to pass the

694 CNS as defined in the challenge scoring rules. The final column indicates the number of

695 points the prediction was given based on the defined scoring criteria.

696 **Supplemental Table 3 - Zhaoping Xiong's method predictive performance on**

697 **regression models.** A summary of the performance of various model architectures

698 tested by top performer Zhaoping Xiong using root-mean-square-error calculated using
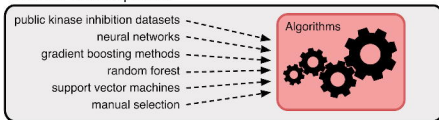
699 true pIC50 values.

700   **Supplemental Table 4 - Top Problem 1 predictions fail to rescue the RET2B fly**

701   **model.** The RET2B model was treated with two positive controls expected to rescue

702   this model (AD80 and APS6-45), a vehicle control (DMSO), and two test compounds

703   (ZINC4090, ZINC9820) predicted by the challenge results to rescue the RET2B model.

704   "tx": the treatment (compound and concentration) used; "genotype": WT or RET2B flies;

705   stage: class of counted animal - adult or pupae; "mean_percent_surv": the mean

706   percent survival across 4 replicates; "std_dev_survival": the standard deviation of the

707   percent survival across 4 replicates.

708   **Supplemental Table 5 - *in silico* chemical modeling of Problem 1 predictions from**

709   **top performers**. Additional hits from top performing teams were characterized using

710   SwissADME to identify compounds with preferred absorption, distribution, metabolism,
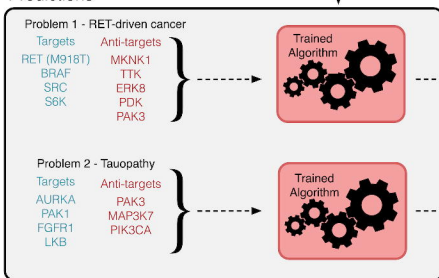
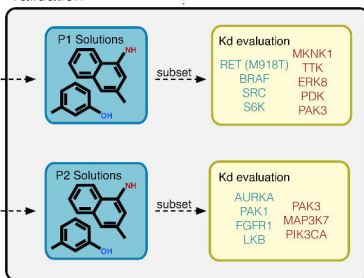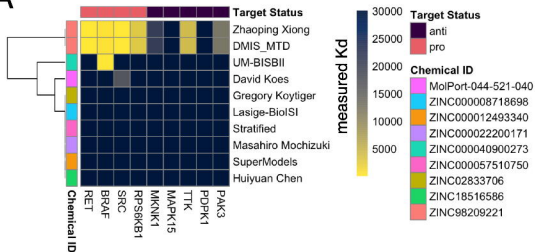711   excretion, and other medicinal chemistry properties.
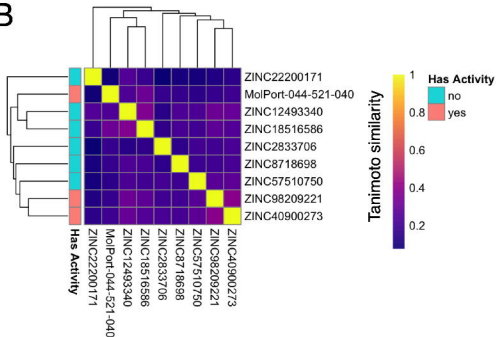
712

Method Development

- public kinase inhibition datasets
- neural networks
- gradient boosting methods
- random forest
- support vector machines
- manual selection

Algorithms

Scoring

P1 K_d

P2 K_d

selectivity
novelty
patentability
drug-like

Top Performers

Predictions

Problem 1 - RET-driven cancer

Targets	Anti-targets
RET (M918T)	MKNK1
BRAF	TTK
SRC	ERK8
S6K	PDK
	PAK3

Trained Algorithm

Problem 2 - Tauopathy

Targets	Anti-targets
AURKA	PAK3
PAK1	MAP3K7
FGFR1	PIK3CA
LKB	

Trained Algorithm

Validation

P1 Solutions

NH

OH

subset

Kd evaluation

RET (M918T)	MKNK1
BRAF	TTK
SRC	ERK8
S6K	PDK
	PAK3

P2 Solutions

NH

OH

subset

Kd evaluation

AURKA	PAK3
PAK1	MAP3K7
FGFR1	PIK3CA
LKB	

A

B

A. Selection of prototype drugs from KIEO

KIEO

Human curation

Search

Search result
RET (M918T)

Sunitinib: POC 9.82
AST487: Kd 4.50
Sorafenib: Kd 7.4
Gefitinib: IC50 0.14

B. Training ReSimNet on the Connectivity Map

Similarity score

ReSimNet: neural network for transcriptional response similarity prediction
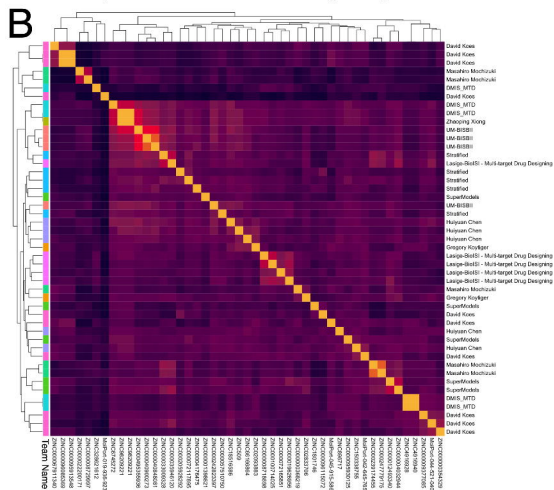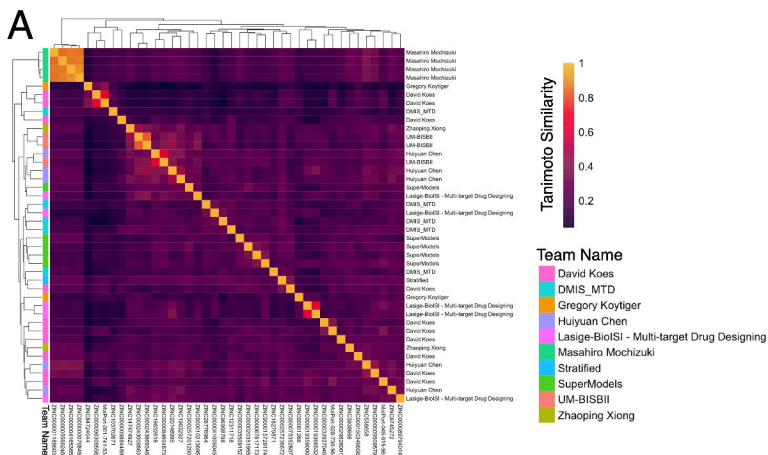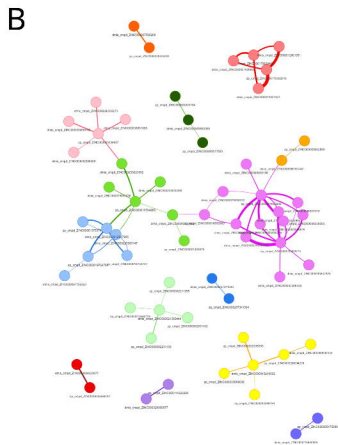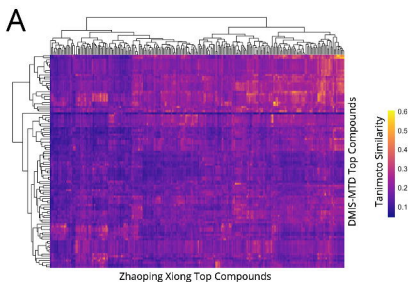
Prototype drug

Trained model
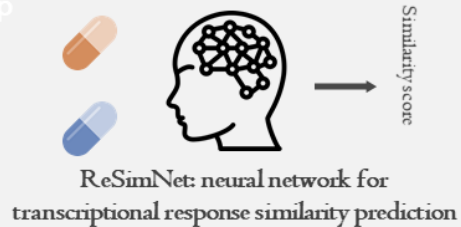
C. Rank compounds from ZINC using ReSimNet and prototype drugs

Prototype drug

ZINC15

Pairs

Predict

Similarity score

0.95

0.73

-0.84

Ranked candidates

Prediction of transcriptional response similarity between a prototype drug and a compound from ZINC15 using ReSimNet and rank aggregation
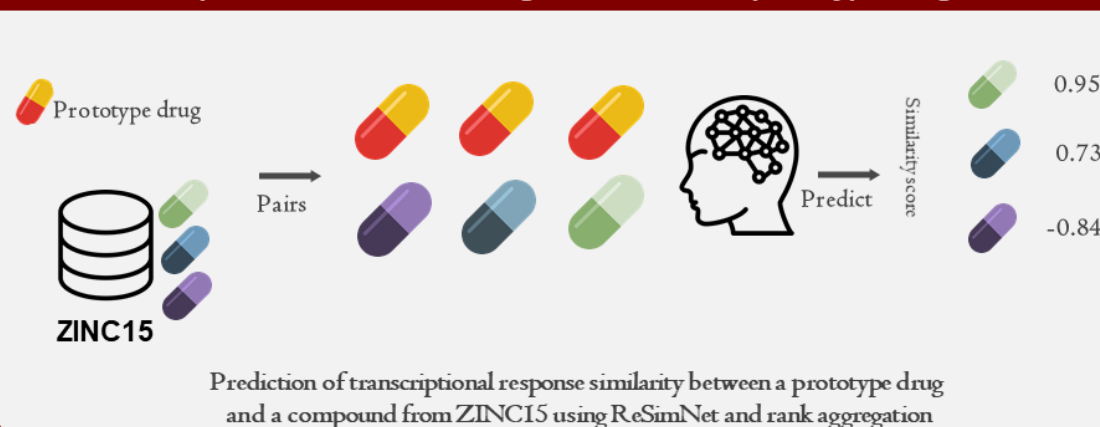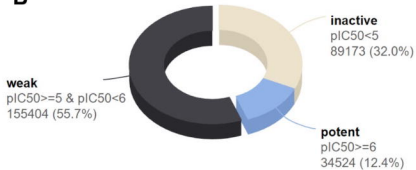
D. Post processing

✓ Lipinski's rules

✓ Patent search

✓ Docking algorithm

A

B

A — Histogram with x-axis "pIC50" (ranging 2 to 10) and y-axis "Counts" (0 to 80000+).

B — Donut chart:
- **inactive** pIC50<5 — 89173 (32.0%)
- **weak** pIC50>=5 & pIC50<6 — 155404 (55.7%)
- **potent** pIC50>=6 — 34524 (12.4%)

C — Confusion matrices:

(80%) Train accuracy: 0.903

true label \ predicted label	inactive	weak	potent
inactive	68415 (0.96)	2445 (0.03)	308 (0.00)
weak	9471 (0.08)	111868 (0.90)	3228 (0.03)
potent	972 (0.04)	5148 (0.19)	21426 (0.78)

(10%) Valid accuracy: 0.849

true label \ predicted label	inactive	weak	potent
inactive	8193 (0.92)	636 (0.07)	83 (0.01)
weak	1516 (0.10)	13313 (0.86)	695 (0.04)
potent	258 (0.07)	1010 (0.29)	2206 (0.64)

(10%) Test accuracy: 0.851

true label \ predicted label	inactive	weak	potent
inactive	8372 (0.92)	620 (0.07)	101 (0.01)
weak	1541 (0.10)	13122 (0.86)	650 (0.04)
potent	223 (0.06)	1016 (0.29)	2265 (0.65)