Integrated Analysis of Drug Sensitivity and Selectivity to Predict Synergistic Drug Combinations and Target Co-addictions in Cancer

Alok Jaiswal[1], Bhagwan Yadav[2], Krister Wennerberg,[1] Tero Aittokallio[1,3]

1. Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland

2. Hematology Research Unit Helsinki (HRUH), University of Helsinki, Finland

3. Department of Mathematics and Statistics, University of Turku, Finland

Correspondence: Tero Aittokallio, email: tero.aittokallio@helsinki.fi

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Abstract

High-throughput drug sensitivity testing provides a powerful phenotypic profiling approach to identify effective drug candidates for individual cell lines or patient-derived samples. Here, we describe an experimental-computational pipeline, named target addiction scoring (TAS), which mathematically transforms the drug response profiles into target addiction signatures, and thereby provides a ranking of potential therapeutic targets according to their functional importance in a particular cancer sample. The TAS pipeline makes use of drug polypharmacology to integrate the drug sensitivity and selectivity profiles through systems-wide interconnection networks between drugs and their targets, including both primary protein targets as well as secondary off-targets. We show how the TAS pipeline enables one to identify not only single-target addictions, but also combinatorial co-addictions among targets that often underlie synergistic drug combinations.

Key words

Precision oncology, Drug sensitivity testing, Drug Polypharmacology, Drug-target interactions, Target addictions, Target deconvolution, Drug combinations

1. Introduction

Recent advances in high-throughput genetic and molecular profiling techniques have enabled an extensive exploration of the genomic landscapes of human tumors, leading to an in-depth understanding of the plethora of genetic alterations present in various cancer types [1-4]. However, these genetic profiling studies have also revealed the massive complexity and heterogeneity across tissue types and even within the same tumor [5]. Furthermore, our limited understanding of the functional consequences of these genetic alterations has posed significant challenges for translating this knowledge into clinically actionable targeted treatment strategies [6]. Cancer cells often exhibit a selective dependence on a few genes or pathways, a phenomenon referred to as 'oncogenic' addiction, which opens up the possibility of exploiting these dependencies for therapeutic developments [7]. However, the extensive genetic heterogeneity results in alterations of multiple molecular pathways, and therefore makes it difficult to pinpoint the specific addictions in particular cancer cells. Complementary strategies are thus needed to identify selective vulnerabilities and cellular addictions of cancer cells toward developing targeted drug combinations that can inhibit the multiple cancer-survival or resistance-driving pathways.

High-throughput compound sensitivity screens that make use of cell-based viability assays to profile the activity of a library of small bioactive molecules (referred hereafter as to 'drugs') have facilitated the identification of promising drug candidates for individual cancer patients (so-called functional precision oncology) [8-12]. The direct advantage of functional drug sensitivity screening assays, compared to genomic and transcriptomic-based profiling, is that they provide a complementary phenotypic information of cancer cells, which is often more clinically actionable. Furthermore, such drug response phenotypic profiles can be used to identify molecular targets or pathway-level addictions based on the knowledge of primary or 'on-targets' of the drugs combined

with their functional response profiles [13-18], a process called ‘target deconvolution’. However, most drugs are promiscuous and lead to a polypharmacological or ‘off-target’ effects [19], which in turn complicate the identification of target addictions directly from the measured drug response phenotypes. Since it is experimentally and economically infeasible to exhaustively explore the full space of all drug-target combinations, there is a need for systematic computational approaches that enable the identification of target addiction patterns from drug sensitivity data.

Here, we describe our experimental-computational target deconvolution pipeline, named target addiction scoring (TAS) [20,21], which makes use of drug polypharmacology to model the complex interconnections between drug responses and target interaction profiles that consider both primary protein targets as well as secondary off-targets. The TAS approach is individualized in the sense that it uses the drug sensitivity profile from a given sample (e.g., cancer cell line or patient-derived sample), screened against a library of bioactive compounds, and then transforms the observed phenotypic profile into a sample-specific target addiction profile, through the information encoded in system-wide drug-target interactions that connects the compounds to their complete spectrum of protein targets (i.e., target selectivity profiles). Systematic mapping of such target addiction signatures provides a ranking of the therapeutic targets according to their functional importance in a given cancer sample. In addition to demonstrating how the TAS approach enables the identification of single-target addictions, we also show here how it can be used to infer combinatorial co-addictions among therapeutic targets, such as kinase pairs, by means of combinatorial TAS approach.

We have previously demonstrated the applicability of TAS by profiling a panel of triple negative breast cancer (TNBC) cell line responses to 239 kinase inhibitors [20,22], in which case the approach was called kinase inhibition sensitivity scoring (KISS). We observed that the KISS

profiles of TNBC cells closely resembled the patient-derived TNBC subtypes, but also revealed novel druggable addictions [20]. However, our approach is not only applicable to cancer cell lines but also to patient-derived cancer samples amenable to high-throughput drug screening [8-10,12]. Moreover, the TAS approach extends naturally to other target classes beyond kinase targets, although the accuracy and coverage of the addiction profiles is critically dependent on the availability of drug-target bioactivity profiles. Drug-target bioactivity data are generally sparse and notoriously heterogeneous due to various experimental assays, and often biased towards specific target classes, such as kinases, and the use of different bioactivity end-points make the implementation of target deconvolution approaches difficult, despite the curated and standardized drug/target databases such as ChEMBL [23]. Therefore, we additionally demonstrate how to use the bioactivity profiles for the purposes of target deconvolution and prediction of drug-target combinations.

2. Materials

The approach makes use of two input data sources, drug sensitivity (Subheading 2.1) and target selectivity (Subheading 2.2) profiles. Drug sensitivity profiling data makes the TAS signatures context-specific for a given cancer cell line or patient sample, whereas the underlying drug-target bioactivity profiles provide the system-wide interactions between drugs and targets that map the potential activity space, independent of the cellular-context.

2.1. Drug sensitivity profiling data

1. A library of targeted compounds is screened against cancer cell lines or patient-derived samples using a high-throughput drug sensitivity profiling assay (*see* **Note 1**). At FIMM, our oncology collection contains both FDA/EMA-approved drugs as well as investigational compounds. Each compound is tested in five increasing concentrations, across a 10,000-fold range, allowing for

the establishment of dose-response curves.

a. We previously tested 21 breast cancer cell lines against 239 anticancer compounds [20,22], using the FIMM drug sensitivity and resistance testing (DSRT) platform [8,12].
b. We have also screened patient-derived cells from acute myeloid leukemia (AML) patients, and used healthy bone marrow samples as controls in the DSRT platform [8,24].

2. One can also use publicly available cancer cell line profiling datasets, such as those from the Sanger institute's Genomics of Drug Sensitivity in Cancer (GDSC) project [14,15], using which we applied the TAS pipeline on a subset of 107 pan-cancer cell lines tested against 138 targeted compounds that included also other drugs than kinase inhibitors. The Broad Institute's Cancer Cell Line Encyclopedia (CCLE)[13] and Cancer Therapeutic Research Portal (CTRP)[16,18] are also useful resources of drug sensitivity data across cancer cell lines, along with their comprehensive genomic and molecular profiles.

2.2. Drug-target bioactivity profiles

1. List of primary 'on-targets' for each compound can be obtained from various resources, such as the targets listed Sanger GDSC (http://www.cancerrxgene.org/); Broad CTRP (http://portals.broadinstitute.org/ctrp/?page=#ctd2BodyHome); DrugBank (https://www.drugbank.ca/) or Drug Gene Interaction Database (DGIdb, http://dgidb.genome.wustl.edu/).

2. For more extensive and quantitative information of drug-target bioactivity profiles for primary, secondary and downstream 'off-target' effects, which are required for the systems-level application of TAS, one can use data from large-scale target selectivity profiling assays, including those for kinase inhibitors [25-27].

3. Comprehensive bioactivity data for many target classes can be retrieved from our publicly available Drug Target Commons (DTC) database (https://drugtargetcommons.fimm.fi/), a crowdsourced platform for bioactivity data annotation, integration, standardization and curation, which also includes assay and bioactivity data from ChEMBL [28,29].

2.3. Software tools/R-packages

1. DSS R-package is available as open-source R implementation at https://bitbucket.org/BhagwanYadav/drug-sensitivity-score-dss-calculation
2. TAS R-package is available as open-source R implementation at https://bitbucket.org/BhagwanYadav/target-addiction-score-tas-calculation.
3. SynergyFinder is available both as web implementation (https://synergyfinder.fimm.fi) and R-package (http://bioconductor.org/packages/release/bioc/html/synergyfinder.html).

3. Methods

The following steps detail the computational methodologies for the TAS approach, both for single-target addiction scoring (Subheading 3.3) and combinatorial TAS (Subheading 3.4).

3.1. Drug Sensitivity Scoring (DSS)

1. Calculate quantitative DSS values by fitting the dose–response curves using four-parameter logistic model [24]. DSS is a closed-form integration of the area under the dose-response curve (AUC), which effectively summarizes the complex dose-response relationship into a single metric (*see* **Note 2**). An open-source R implementation of DSS package is made available for this purpose (see Section 2.3).
2. Calculate differential DSS (dDSS) by comparing the DSS of cancer sample to that of the

controls (*see* **Note 3**). Differential DSS allows one to estimate the selective response of a given cancer sample to the particular drug, compared to the control background response distribution, which is eventually useful when quantifying the selective target addictions of the particular cancer samples.

3.2. Drug-target Interactions

1. Extract the quantitative drug-target interaction bioactivity profiles of compounds from the DTC web portal (https://drugtargetcommons.fimm.fi/).
2. Define the potent targets of a particular compound by using all the available bioactivity data for the compound and a suitable activity threshold:
   a) If there are enough dose-response bioactivity end-points (K_d, K_i or IC_{50}) for the compound (say, for more than 20 distinct proteins), define the potent targets as those with less than 50-fold bioactivity compared to the smallest bioactivity value over all the proteins (this typically corresponds to the 'on target'). In case of limited number of data points, define potent targets as those with K_d, K_i or $IC_{50} \leq 100$ nM (*see* **Note 4**).
   b) For the activity measurements (activity%, residual activity%, %inhibition or potency), often resulting from assays with only single or a few concentration points, define a more stringent threshold: ≤10% residual activity (≥90% inhibition) for the test concentration of ≤1000 nM and ≤20% for a test concentration of ≤500 nM in biochemical assays. For cell-based assays, set the threshold to ≤50% residual activity for the test concentration of ≤1000 nM and ≤10% for the test concentration ≤10000 nM.

3.3. Target Addiction Scoring (TAS)

The TAS value provides an estimate of the sensitivity of a cell to the inhibition of a particular target. More specifically, for a given target t, TAS_t is calculated by averaging the observed drug

response (e.g., DSS_i) (*see* **Note 2**) over all those n_t inhibitors (i) that target the protein t (Figure 1A). Mathematically, TAS defines a transformation between the spaces spanned by the compounds and their targets, which maps observed drug responses to the underlying target addictions:

$$TAS_t = \sum_{i=1}^{n_t} \frac{DSS_i}{n_t}$$

1. Calculate the single-target TAS for each sample separately and sort the targets based on the increasing TAS values, which enables one to prioritize the pharmacologically actionable target addictions in individual cancer samples for further pre-clinical validation (*see* **Note 6**). An open-source R implementation of TAS package is made available for this purpose (see Section 2.3).
2. Determine the statistical significance of a TAS value empirically using permutation testing; based on a vector of inhibitors per target, randomly select a given number of inhibitors, and then average their DSS values in a given sample. The permutation procedure is repeated, simulating at least 10,000 random TAS values in the given sample. The empirical p-value is defined by the percentage of the permuted TAS values above or equal to the observed one.

3.4. Prediction of synergistic target pairs

1. For all those target pairs (t_1, t_2) that are inhibited by the same compound, calculate a combinatorial TAS score by averaging the drug response (e.g., DSS) over the set of common inhibitors that target both t_1 and t_2. Target pairs that have no common inhibitors in the library are excluded from the combinatorial TAS analysis.
2. Calculate complement scores for each target in a target pair. The complement score (CS) of t_1 or t_2 is defined as the average response (e.g., DSS) over the inhibitors that inhibit only t_1 or

$t_{2,}$ respectively, but not both of the targets (see Figure 1B for a schematic illustration of the complement score concept).

3. Exclude those target pairs for which the difference between the combinatorial TAS and the complement scores is below a selected cut-off value (e.g, $T = 4$). Rank the remaining target pairs by the magnitude of their combinatorial TAS, and map back to drug pairs by selecting the strongest inhibitors of targets t_1 and t_2 alone, excluding their common inhibitors, using the lowest bioactivity values.
4. Experimentally validate the top co-addicted target pairs predicted by the combinatorial TAS, e.g, by testing the inhibitors in an 8 × 8 dose-matrix format covering seven increasing concentrations of each drug, along with all their pairwise combinations [20,30] (see Figure 2). Evaluate the degree of synergy between the pairwise combination effects using the synergy scoring models implemented in the SynergyFinder package [31] (*see* **Note 7**).

4. Notes

1. The DSRT platform uses of a library of compounds dissolved in 100% dimethyl sulfoxide (DMSO) and dispensed on tissue culture treated 384-well plates using an acoustic liquid handling device. The compounds are plated at 5 different concentrations in 10-fold dilutions covering a 10,000-fold concentration range, centered around a compound-specific relevant cellular activity concentration (e.g. 1–10,000 nM for a compound with an on-target cellular half-maximal effect of about 100 nM). The pre-drugged plates are stored in pressurized Storage Pods filled with inert nitrogen gas. For the assay, the compounds are first dissolved with 5 µl of culture medium while shaking for 30 min, 20 µl of single cell suspension (10,000 cells) is then seeded to each well using a MultiDrop Combi peristaltic dispenser. The plates are incubated in a humidified environment at 37°C and 5% CO_2 and after 72 h cell viability was measured using CellTiter-Glo luminescent assay (Promega),

according to manufacturer's instructions. The data are normalized to negative control (DMSO only) and positive control wells (100 μM benzethonium chloride).

2. In addition to the drug sensitivity score (DSS), a number of other metrics, such as the area under the drug response (AUC) and half-maximal inhibitory concentration (IC_{50}), are also frequently used for summarizing dose-response curves and quantifying drug sensitivities. In our studies, we have mainly used DSS as the response metric, as it has been shown to provide robust and reproducible drug response profiles [24,32].
3. High-throughput drug sensitivity screening is amenable to profiling of a compendium of patient-derived cells or cancer cell lines, making it possible also to study the variability of drug response across different samples to the same drug. Whenever possible, we calculate the differential DSS (dDSS) values in each patient sample by subtracting the mean DSS for a drug across all healthy controls from the observed DSS of a given cancer sample. However, sometimes it is difficult to define the control responses for cancer cell lines; in our previous work on breast cancer cell lines [20], we used the DSS of MCF10A cells as a control to calculate the differential DSS. Alternatively, we have also used the average DSS across all the samples screened against a given drug for calculating dDSS [21,22].
4. Defining potent on- and off-targets for a compound may not be straightforward, especially in cases where only a few bioactivity values are available from target selectivity profiling studies. Further challenges originate from differences in the bioactivity assays. For instance, biochemical assays typically generate higher potency levels than cell-based assays, and therefore require more stringent cut-off values. Dose-response bioactivity end-points are often more reliable than those based on single or a few concentration points, and therefore K_d, K_i and IC_{50} end-points are preferred [33]. In cases where there are multiple bioactivity values for the same compound-target pair, originating from different studies or other data resources, one can take median bioactivity value that is more robust to outliers than the

mean value.

5. We manually filter out targets involved in cellular metabolism, such as cytochrome (CYP) and ATP-binding cassette (ABC) genes, from the set of potential drug targets when analyzing *in vitro* drug responses, since those are unlikely to contribute to the drug's mode of action in cell line drug testing.
6. It is also possible to incorporate information from biological pathways and signaling networks for identifying target addictions of proteins that are upstream or downstream of a given drug target. Often, one will find drugs that show selective drug response in a cancer sample, even if their direct targets might not show selective addiction. Therefore, additional molecular profiling datasets such as transcriptomic, proteomic and genome-wide loss-of-function RNAi or CRISPR screens, that provide complementary target validation information, can be used to identify targets that mechanistically explain the observed drug responses and also serves as a pre-clinical validation of the hits from the TAS approach.
7. For drug combination synergy scoring, we have previously made available the SynergyFinder web implementation (https://synergyfinder.fimm.fi) and R-package (http://bioconductor.org/packages/release/bioc/html/synergyfinder.html) to calculate the drug synergy scores using different reference models, including Zero Interaction Potency (ZIP), Bliss and Loewe models, as well as the Highest Single Agent (HSA) [31]. Sometimes it may be convenient to calculate the synergy scores using various models to come up with the most reliable determination of the degree of synergy for a given drug pair [34].

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References:

1. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW (2013) Cancer Genome Landscapes. Science 339 (6127):1546

2. Garraway Levi A, Lander Eric S (2013) Lessons from the Cancer Genome. Cell 153 (1):17-37

3. The Cancer Genome Atlas Research N (2017) Integrated genomic and molecular characterization of cervical cancer. Nature 543 (7645):378-384

4. The Cancer Genome Atlas Research N (2017) Integrated genomic characterization of oesophageal carcinoma. Nature 541 (7636):169-175

5. Marusyk A, Almendro V, Polyak K (2012) Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12 (5):323-334

6. Yi S, Lin S, Li Y, Zhao W, Mills GB, Sahni N (2017) Functional variomics and network perturbation: connecting genotype to phenotype in cancer. Nat Rev Genet 18 (7):395-410

7. Weinstein IB, Joe A (2008) Oncogene Addiction. Cancer Research 68 (9):3077

8. Pemovska T, Kontro M, Yadav B, Edgren H, Eldfors S, Szwajda A, Almusa H, Bespalov MM, Ellonen P, Elonen E, Gjertsen BT, Karjalainen R, Kulesskiy E, Lagström S, Lehto A, Lepistö M, Lundán T, Majumder MM, Lopez Marti JM, Mattila P, Murumägi A, Mustjoki S, Palva A, Parsons A, Pirttinen T, Rämet ME, Suvela M, Turunen L, Västrik I, Wolf M, Knowles J, Aittokallio T, Heckman CA, Porkka K, Kallioniemi O, Wennerberg K (2013) Individualized Systems Medicine (ISM) strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia. Cancer Discovery 3 (12):1416-1429

9. Pemovska T, Johnson E, Kontro M, Repasky GA, Chen J, Wells P, Cronin CN, McTigue M, Kallioniemi O, Porkka K, Murray BW, Wennerberg K (2015) Axitinib effectively inhibits BCR-ABL1(T315I) with a distinct binding conformation. Nature 519 (7541):102-105

10. Tyner JW, Yang WF, Bankhead A, Fan G, Fletcher LB, Bryant J, Glover JM, Chang BH, Spurgeon SE, Fleming WH, Kovacsovics T, Gotlib JR, Oh ST, Deininger MW, Zwaan CM, Den Boer ML, van den Heuvel-Eibrink MM, Hare T, Druker BJ, Loriaux MM (2013) Kinase Pathway Dependence in Primary Human Leukemias Determined by Rapid Inhibitor Screening. Cancer Research 73 (1):285

11. Friedman AA, Letai A, Fisher DE, Flaherty KT (2015) Precision medicine for cancer with next-generation functional diagnostics. Nat Rev Cancer 15 (12):747-756

12. Malani D, Murumagi A, Yadav B, Kontro M, Eldfors S, Kumar A, Karjalainen R, Majumder MM, Ojamies P, Pemovska T, Wennerberg K, Heckman C, Porkka K, Wolf M, Aittokallio T, Kallioniemi O (2017) Enhanced sensitivity to glucocorticoids in cytarabine-resistant AML. Leukemia 31 (5):1187-1195

13. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehar J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jane-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway A, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P, de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winckler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Morrissey MP, Sellers WR, Schlegel R, Garraway LA (2012) The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483 (7391):603-307

14. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, Greninger P, Thompson IR, Luo X, Soares J, Liu Q, Iorio F, Surdez D, Chen L, Milano RJ, Bignell GR, Tam AT, Davies H, Stevenson JA, Barthorpe S, Lutz SR, Kogera F, Lawrence K, McLaren-Douglas A, Mitropoulos X, Mironenko T, Thi H, Richardson L, Zhou W, Jewitt F, Zhang T, O/'Brien P, Boisvert JL, Price S, Hur W, Yang W, Deng X, Butler A, Choi HG, Chang JW, Baselga J, Stamenkovic I, Engelman JA, Sharma SV, Delattre O, Saez-Rodriguez J, Gray NS, Settleman J, Futreal PA, Haber DA, Stratton MR, Ramaswamy S, McDermott U, Benes CH (2012) Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483 (7391):570-575

15. Iorio F, Knijnenburg TA, Vis DJ, Bignell GR, Menden MP, Schubert M, Aben N, Gonçalves E, Barthorpe S, Lightfoot H, Cokelaer T, Greninger P, van Dyk E, Chang H, de Silva H, Heyn H, Deng X, Egan RK, Liu Q, Mironenko T, Mitropoulos X, Richardson L, Wang J, Zhang T, Moran S, Sayols S, Soleimani M, Tamborero D, Lopez-Bigas N, Ross-Macdonald P, Esteller M, Gray NS, Haber DA, Stratton MR, Benes CH, Wessels LFA, Saez-Rodriguez J, McDermott U, Garnett MJ A Landscape of Pharmacogenomic Interactions in Cancer. Cell 166 (3):740-754

16. Basu A, Bodycombe Nicole E, Cheah Jaime H, Price Edmund V, Liu K, Schaefer Giannina I, Ebright Richard Y, Stewart Michelle L, Ito D, Wang S, Bracha Abigail L, Liefeld T, Wawer M, Gilbert Joshua C, Wilson Andrew J, Stransky N, Kryukov Gregory V, Dancik V, Barretina J, Garraway Levi A, Hon CS-Y, Munoz B, Bittker Joshua A, Stockwell Brent R, Khabele D, Stern Andrew M, Clemons Paul A, Shamji Alykhan F, Schreiber Stuart L An Interactive Resource to Identify Cancer Genetic and Lineage Dependencies Targeted by Small Molecules. Cell 154 (5):1151-1161

17. Rees MG, Seashore-Ludlow B, Cheah JH, Adams DJ, Price EV, Gill S, Javaid S, Coletti ME, Jones VL, Bodycombe NE, Soule CK, Alexander B, Li A, Montgomery P, Kotz JD, Hon CS-Y, Munoz B, Liefeld T, Dancik V, Haber DA, Clish CB, Bittker JA, Palmer M, Wagner BK, Clemons

PA, Shamji AF, Schreiber SL (2016) Correlating chemical sensitivity and basal gene expression reveals mechanism of action. Nat Chem Biol 12 (2):109-116

18. Seashore-Ludlow B, Rees MG, Cheah JH, Cokol M, Price EV, Coletti ME, Jones V, Bodycombe NE, Soule CK, Gould J, Alexander B, Li A, Montgomery P, Wawer MJ, Kuru N, Kotz JD, Hon CS-Y, Munoz B, Liefeld T, Dančík V, Bittker JA, Palmer M, Bradner JE, Shamji AF, Clemons PA, Schreiber SL (2015) Harnessing Connectivity in a Large-Scale Small-Molecule Sensitivity Dataset. Cancer Discovery 5 (11):1210-1223

19. Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol 4 (11):682-690

20. Szwajda A, Gautam P, Karhinen L, Jha Sawan K, Saarela J, Shakyawar S, Turunen L, Yadav B, Tang J, Wennerberg K, Aittokallio T (2015) Systematic Mapping of Kinase Addiction Combinations in Breast Cancer Cells by Integrating Drug Sensitivity and Selectivity Profiles. Chemistry & Biology 22 (8):1144-1155

21. Yadav B, Gopalacharyulu P, Pemovska T, Khan SA, Szwajda A, Tang J, Wennerberg K, Aittokallio T (2015) From drug response profiling to target addiction scoring in cancer cell models. Disease Models & Mechanisms 8 (10):1255-1264

22. Gautam P, Karhinen L, Szwajda A, Jha SK, Yadav B, Aittokallio T, Wennerberg K (2016) Identification of selective cytotoxic and synthetic lethal drug responses in triple negative breast cancer cells. Molecular Cancer 15 (1):34

23. Hersey A, Chambers J, Bellis L, Patrícia Bento A, Gaulton A, Overington JP (2015) Chemical databases: curation or integration by user-defined equivalence? Drug Discovery Today: Technologies 14:17-24

24. Yadav B, Pemovska T, Szwajda A, Kulesskiy E, Kontro M, Karjalainen R, Majumder MM, Malani D, Murumägi A, Knowles J, Porkka K, Heckman C, Kallioniemi O, Wennerberg K, Aittokallio T (2014) Quantitative scoring of differential drug sensitivity for individually optimized anticancer therapies. Scientific Reports 4:5193

25. Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, Hocker M, Treiber DK, Zarrinkar PP (2011) Comprehensive analysis of kinase inhibitor selectivity. Nat Biotech 29 (11):1046-1051

26. Metz JT, Johnson EF, Soni NB, Merta PJ, Kifle L, Hajduk PJ (2011) Navigating the kinome. Nat Chem Biol 7 (4):200-202

27. Knapp S, Arruda P, Blagg J, Burley S, Drewry DH, Edwards A, Fabbro D, Gillespie P, Gray NS, Kuster B, Lackey KE, Mazzafera P, Tomkinson NCO, Willson TM, Workman P, Zuercher WJ (2013) A public-private partnership to unlock the untargeted kinome. Nat Chem Biol 9 (1):3-6

28. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, Krüger FA, Light Y, Mak L, McGlinchey S, Nowotka M, Papadatos G, Santos R, Overington JP (2014) The ChEMBL bioactivity database: an update. Nucleic Acids Research 42 (D1):D1083-D1090

29. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B, Overington JP (2012) ChEMBL: a large-scale bioactivity database for drug discovery. Nucleic Acids Research 40 (D1):D1100-D1107

30. He L, Kulesskiy E, Saarela J, Turunen L, Wennerberg K, Aittokallio T, Tang J (2016) Methods for High-Throughput Drug Combination Screening and Synergy Scoring. bioRxiv. doi:10.1101/051698

31. Ianevski A, He L, Aittokallio T, Tang J (2017) SynergyFinder: a web application for analyzing drug combination dose–response matrix data. Bioinformatics. doi:10.1093/bioinformatics/btx168

32. Mpindi JP, Yadav B, Östling P, Gautam P, Malani D, Murumägi A, Hirasawa A, Kangaspeska S, Wennerberg K, Kallioniemi O, Aittokallio T (2016) Consistency in drug response profiling. Nature 540 (7631):E5-E6

33. Santos R, Ursu O, Gaulton A, Bento AP, Donadi RS, Bologa CG, Karlsson A, Al-Lazikani B, Hersey A, Oprea TI, Overington JP (2017) A comprehensive map of molecular drug targets. Nat Rev Drug Discov 16 (1):19-34

34. Tang J, Wennerberg K, Aittokallio T (2015) What is synergy? The Saariselkä agreement revisited. Frontiers in Pharmacology 6:181

35. Bliss CI (1939) The toxicity of poisons applied jointly. Annals of Applied Biology 26 (3):585-615

Figure Legends:

Figure 1: Schematic illustration of the single and combinatorial target addiction scoring (TAS). (A) Left: Single TAS approach ranks each target (*t*) in the context of a given drug-target network based on the average drug sensitivity score (DSS) over the subset of its potent inhibitors (*n*). Single TAS enables one to prioritize pharmacologically actionable kinase signal addictions in individual cancer cell samples for experimental validation. Right: The TAS concept was extended to ranking target pairs (t_a, t_b) based on their average combinatorial effect over the subset of inhibitors targeting both of the kinases (*m*). Combinatorial TAS enables one to identify synthetic lethal type of target pairs, which may correspond to synergistic drug combinations between their inhibitors. Experimental validation of the combinatorial TAS predictions can be carried out by testing the most potent inhibitors of t_a and t_b in combination assays.

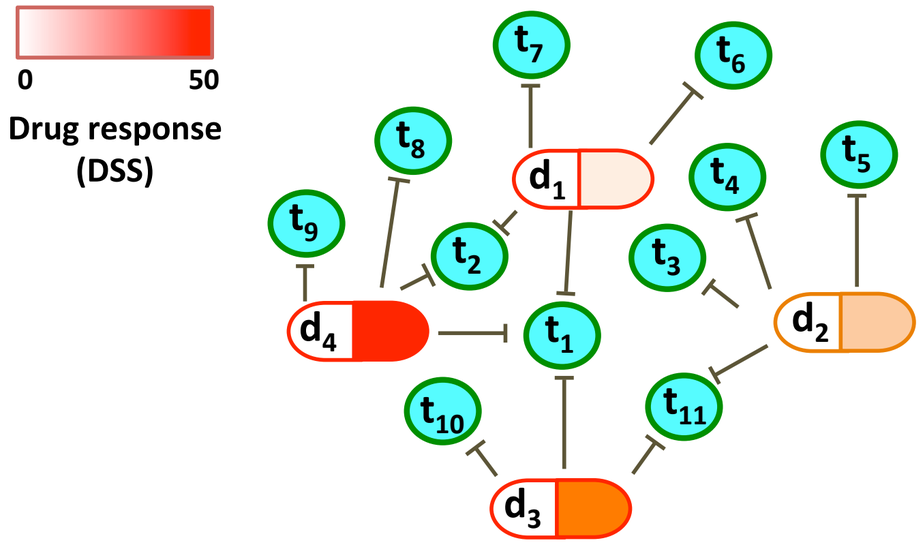
(B) Relationships between the single and combinatorial TAS defined using set-theoretic operations among the set of inhibitors. Using this notation, the cardinality of the set *A* is *n* and the cardinality of the intersection between *A* and *B* is *m*. The complement score (CS) of t_a or t_b is defined as the average drug response over the inhibitors that belong to the difference between sets *A* and *B* or *B* and *A*, respectively (the shaded portions) (modified from ref. 20 with permission from Elsevier).

Figure 2: Combinatorial TAS predicts synergistic drug combinations and co-addicted target pairs. (A) Dose-response curves for the predicted synergistic effect between axitinib and dasatinib on cell viability in HCC1937 human breast cancer cells. The red dose-response curve shows the observed combination effect between axitinib (at multiple concentrations, y-axis) and dasatinib (at fixed 100 nM concentration; see Figure 2B, highlighted column). The black curve represents the dose-response to axitinib only. The blue curve represents the expected dose-response of axitinib (at

multiple concentrations) combined with dasatinib (at fixed 100 nM concentration). The expected combinatorial effects were calculated based on the Bliss independence model [35]. Each combination was tested in two to four replicates. Points and error bars represent the mean and its standard error (SEM), respectively, and the solid curve is the logistic function fit.

(B) An example of the Bliss excess scores matrix for the synergistic combination effects between axitinib and dasatinib (see Figure 2A). The values in the matrix are synergy scores calculated using the Bliss independence model at each tested combinatorial concentration of the two drugs (the first row and column of the matrix show the single-drug dose-response curves, where the other drug is at zero concentration). The highlighted column (dasatinib 100nM) corresponds to the red curve in Figure 2A (reproduced from ref. 20 with permission from Elsevier).

A



Drug – target interaction network

Single TAS

$$TAS(t_a) = \frac{1}{n}\sum_{d_i \to k} DSS_i$$

Rank individual targets in each sample

Combinatorial TAS

$$TAS(t_a, t_b) = \frac{1}{m}\sum_{d_i \to t_a, t_b} DSS_i$$

Filtering:

$$TAS(t_a, t_b) - max\{CS(t_a), CS(t_b)\} > T$$

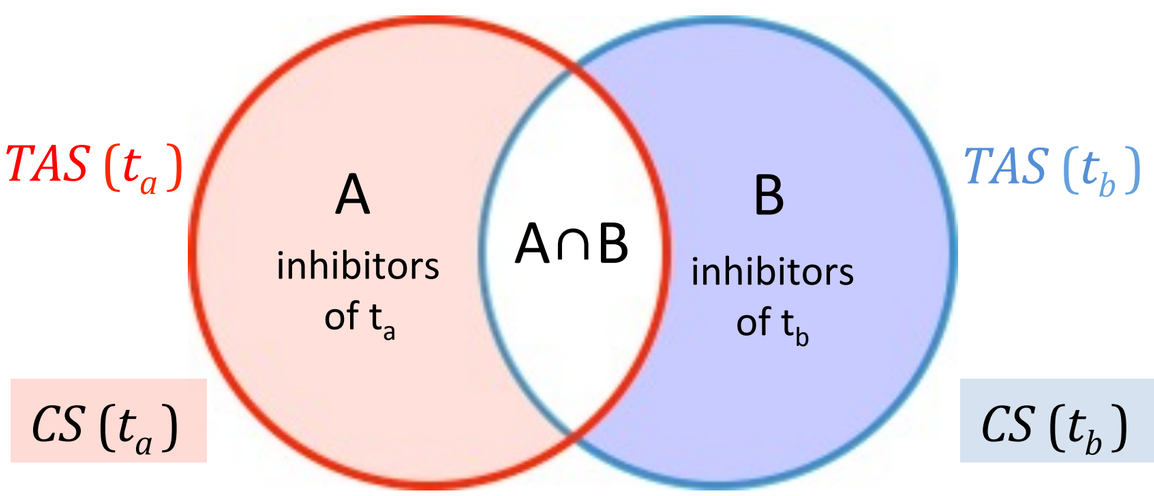
Rank all target pairs in each sample

Strongest inhibitor of t_a
+
Strongest inhibitor of t_b
} Drug combination

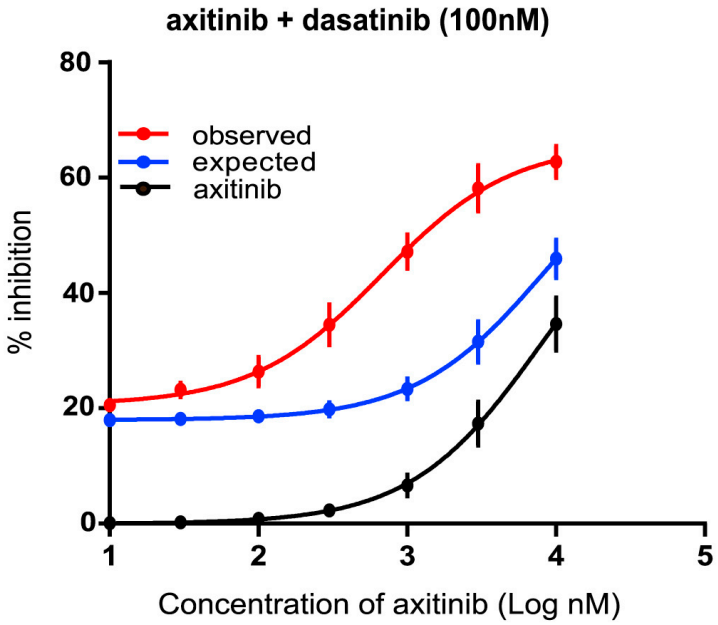
B

$$TAS\,(t_a|\mathrm{A}) = TAS\,(t_a, t_b|\mathrm{A}\cap\mathrm{B}) + CS\,(t_a|\mathrm{A}\backslash\mathrm{B})$$

$n = |\mathrm{A}|$, $m = |\mathrm{A}\cap\mathrm{B}|$



A



B

dasatinib (nM)

axitinib (nM)	0	1	3	10	30	100	300	1000
0								
10		0.00	0.00	0.01	0.04	0.05	-0.06	0.04
30		0.00	0.01	0.02	0.05	0.08	-0.04	0.03
100		0.00	0.01	0.02	0.06	0.11	-0.02	0.04
300		0.00	0.00	0.01	0.04	0.21	0.09	0.03
1000		0.03	0.04	0.08	0.18	0.31	0.12	0.10
3000		0.10	0.11	0.17	0.28	0.37	0.15	0.13
10000		0.09	0.10	0.12	0.16	0.20	0.09	