OncoStratifier: Stratifying Oncogene-addicted Cohorts By Drug Response

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Abstract

Oncogenes, when mutated or overexpressed, drive tumorigenesis and lead to oncogene addiction, where cancer cells rely on these genes for survival and proliferation. Stratifying oncogene-addicted cohorts is essential for precision medicine but remains underexplored. We propose OncoStratifier, a framework to identify drugs that specifically target oncogene-addicted cancer cells, differentiating between those that induce sensitivity or resistance. Our results reveal 21,020 stratifying drugs across 267 oncogenes in 31 different cancer types. We have identified 59 mutational markers associated with 36 of these stratifying drugs across 36 distinct oncogenes and 11 cancer types. These findings underscore OncoStratifier's potential to guide personalized cancer treatment strategies.

Keywords: oncogene, patient stratification, oncogene addiction, drug response

1 Introduction

Cancer is characterized by a complex interplay of genetic and environmental factors that drive uncontrolled cell proliferation and survival, hallmarks of the disease (1–3). Central to this process are oncogenes, which are mutated or overexpressed versions of normal genes (proto-oncogenes) that contribute to tumorigenesis (4). Oncogenes such as KRAS (5), EGFR (6), and MYC (7) play pivotal roles in regulating cell growth, division, and differentiation. When these genes are altered, they can lead to oncogene addiction, where cancer cells become heavily reliant on the continuous activity of these oncogenes for their survival and proliferation (4).

Oncogene addiction provides a therapeutic target as cancer cells dependent on these oncogenes can be selectively targeted by drugs that inhibit their function. For example, inhibitors targeting BCR-ABL in chronic myeloid leukemia (CML) (8) or EGFR inhibitors in non-small cell lung cancer (NSCLC) (9) have shown significant clinical success. However, not all oncogene-addicted cancers can be effectively targeted. Some oncogenes are challenging to inhibit directly due to their structural properties, while others develop resistance to targeted therapies through secondary mutations or activation of alternative signaling pathways (10,11). KRAS, for example, lacks deep binding pockets and has a high affinity for GTP/GDP, making it difficult to develop effective inhibitors (12). These resistance mechanisms often result from the dynamic changes in cancer cell characteristics, leading to variable drug sensitivities and the need for novel therapeutic strategies

Patient stratification is a critical component of precision medicine, aiming to optimize treatment outcomes by tailoring therapies to individual patient profiles. Traditionally, stratification has been performed either on the entire cancer patient population or within specific cancer types, delineating subtypes based on genetic, molecular, and clinical features (13). While these approaches have improved treatment efficacy and patient survival, they often overlook the unique and specific characteristics of oncogene-addicted cohorts.

Given the distinctive nature of oncogene addiction and the associated treatment challenges, there is a pressing need to stratify these cohorts with high resolution. Stratifying oncogene-addicted patients who currently lack effective treatments can uncover specific vulnerabilities and guide the development of more targeted and effective therapies. Despite its potential, such stratification has not been systematically implemented.

To address this gap, we propose the OncoStratifier framework, a systematic approach to identify drugs that specifically stratify oncogene-addicted cohorts. This framework aims to uncover drugs that can effectively target these unique patient populations by examining changes in drug response specific to oncogene addiction. OncoStratifier categorizes drugs based on whether they induce sensitivity or resistance within the oncogene-addicted cohort, ensuring that the observed effects are truly specific to the oncogene in question.

By employing this framework, we can better understand the landscape of drug responses in oncogene-addicted cancers and identify promising therapeutic candidates. This approach not only

enhances our ability to provide effective treatments for oncogene-addicted patients but also contributes to the broader field of precision oncology, where the goal is to deliver the right treatment to the right patient at the right time. Notably, OncoStratifier identified 21,020 instances of exclusive stratification in oncogene-addicted cohorts, highlighted increased sensitivity in KRAS-addicted colorectal cancer and EGFR-addicted cohorts, and revealed 59 mutational markers associated with 36 stratifying drugs across 11 cancer types, providing valuable insights for personalized treatment strategies.

2 Results

2.1 Oncogenic addiction influences the response to targeted therapy

In the OncoStratifier framework, drugs demonstrating significant impact were categorized into four groups based on their stratifying characteristics and changes in response rates (sensitivity) between mutated and wild-type cohorts. While *stratifying* categories refer to oncogene-mutated cohort being exclusively stratified by drug response, artifact *full* categories refer to oncogene-wt cohort being exclusively stratified. Stratifying sensitivity (*SS*) encompasses drugs where the mutated cohort is stratified by gaining sensitivity compared to the wild-type (WT) cohort. In contrast, stratifying resistance (*SR*) refers to drugs where the number of responsive cell lines in the mutated cohort decreases, indicating a gain in resistance while resulting in a stratified cohort. Additionally, artifact categories include full sensitivity (*FS*), where the mutated cohort gains sensitivity, and full resistance (*FR*), where it loses sensitivity, with the latter two categories primarily stratifying the WT cohort (**Figure 1a**).

We identified 21,020 instances where drugs exclusively stratified the oncogene addicted cohort, comprising 13,094 *SS* and 7,926 *SR* cases across 267 oncogenes in 31 different cancer types (**Figure *1*b, Supplementary Table S1-S2)**. While most significant drugs exhibited near maximal changes in stratification ability (Oncostratifier Score≈1 or ≈-1), there were notable exceptions with minimal but statistically significant changes (Oncostratifier Score≈0).

Further examination focused on prominent oncogene addictions, such as KRAS and EGFR in colorectal carcinoma (**Figure *1*c-d**). KRAS mutations, prevalent in various cancers, pose challenges in direct targeting due to the complexity of its binding site and high affinity for downstream effectors, often leading to off-target effects (14). We identified three stratifying drugs where the KRAS-WT cohort was

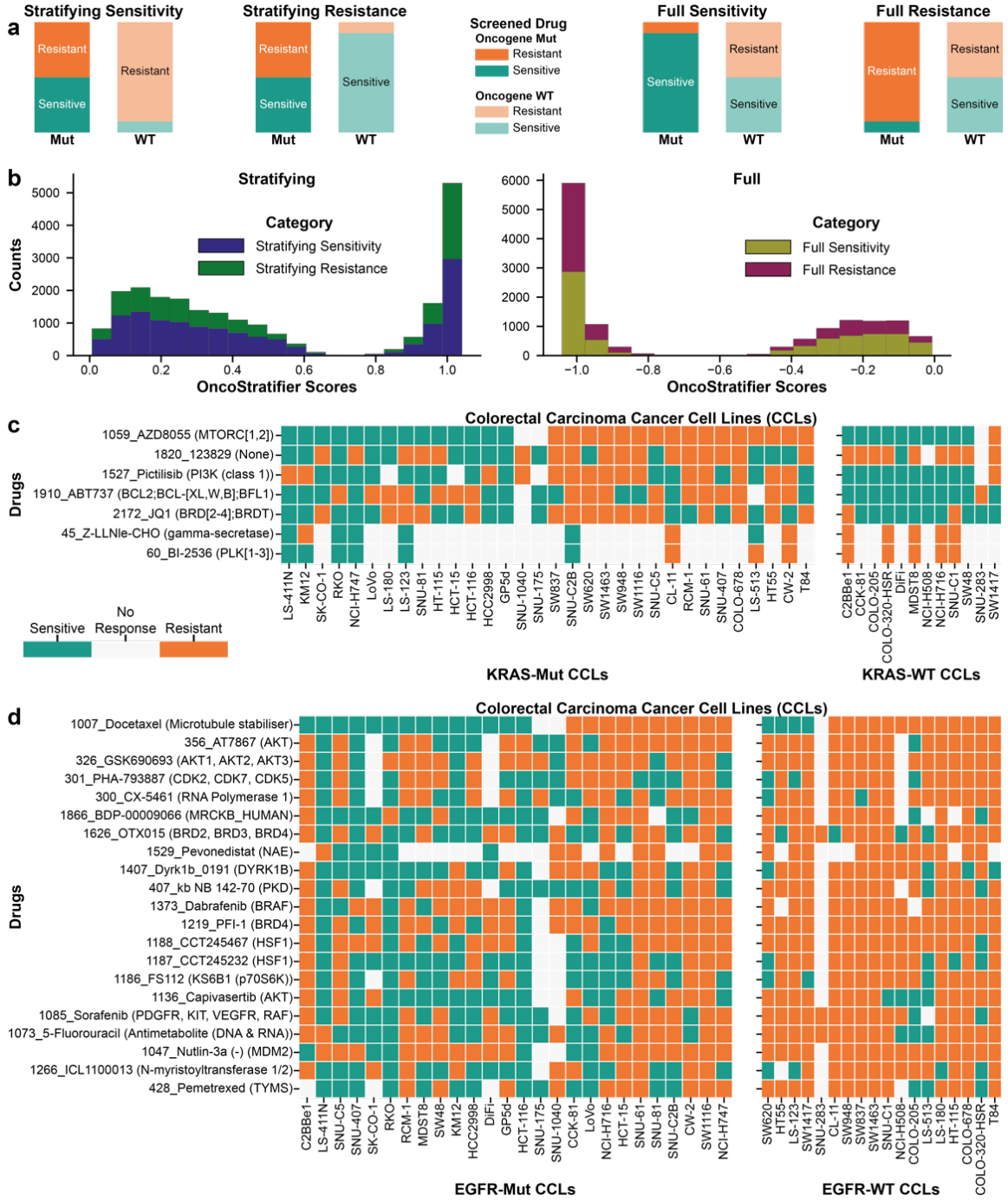

Figure 1 Oncostratifier categories, distribution of significant drugs, and KRAS & EGFR addictions in colorectal carcinoma. a, 4 categories of drugs found by Oncostratifier: Stratifying categories include drugs that exclusively stratify the oncogenic addicted cohort whereas Full categories include drugs which exclusively stratifies oncogene-WT cohort. On the other hand, Sensitivity categories refer to oncogene addicted cohort gaining sensitivity to selected drug whereas Resistance categories refer to drugs gaining sensitivity with oncogene addiction. **b,** Histogram of drugs found significantly per Oncostratifier category. **c-d,** Drug response of cancer cell lines to stratifying drugs found in KRAS **(c)** or EGFR **(d)** addiction in colorectal carcinoma divided by oncogene addicted and oncogene-WT cohorts.

predominantly resistant, yet the KRAS-Mut cohort showed increased sensitivity, attributed to KRAS addiction: *123829*, *Z−LLNle−Cho*, and *BI−2536*. One of these drugs, the gamma secretase inhibitor

(GSI) *Z−LLNle−Cho,* interferes with Notch signaling (15) which is required for the survival of KRAS induced lung cancer cells (16). This finding highlights the sensitivity gain associated with KRAS mutations, although observed in a different cancer type. Conversely, drugs like *AZD8055*, *Pictilisib*, *ABT737*, and *JQ1* demonstrated increased resistance in KRAS-Mut cohorts. Drugs found in both categories underscore the intricate relationship between genetic mutations and drug response, which could inform more targeted treatment approaches and could be a potential therapy for KRAS-addicted patients.

EGFR (Epidermal Growth Factor Receptor) is a well-documented oncogene in various cancers, including colorectal cancer (17–19). Mutational activation of EGFR leads to uncontrolled cell proliferation and survival. EGFR's role in colorectal cancer makes it a prime target for anticancer therapies, with several anti-EGFR therapies such as cetuximab (20,21) and panitumumab (22) already demonstrating clinical efficacy in managing disease progression in patients with wild-type KRAS (23,24). However, the development of resistance to EGFR-targeted therapies, often through secondary mutations or alternative signaling pathways (20,24), remains a challenge, underscoring the need for identifying additional drugs that can bypass or overcome these resistance mechanisms.

In our analysis, we identified 21 drugs that stratify the EGFR-Mut cohort with increased sensitivity (*SS*), indicating a potential for these drugs to enhance treatment outcomes in patients exhibiting EGFR-driven oncogenesis. 5 of these drugs; *pevonedistat*, *PFI-1*, *GSK690693*, *nutlin-3a (-)*, and *dabrafenib* have an almost maximum score of 1 which suggests that they nearly perfectly stratify the EGFR-addicted patients while all EGFR-WT cancer cell lines are fully resistant to the drug. The observed sensitivity to the NEDD8 inhibitor *pevonedistat* in EGFR-mutated colorectal cancer cell lines is corroborated by literature demonstrating that the combined blockade of NEDD8 and EGFR pathways significantly enhances growth arrest and apoptosis in colorectal cancer models (25).

In total, we identified stratifying drugs for 164 different oncogene addictions where the relation between the oncogene and cancer is supported by literature including KRAS and EGFR colorectal carcinoma, ERBB2 in breast carcinoma, MYC in ovarian carcinoma (Supplementary File) The drugs identified in both *SS* and *SR* categories illustrate the complex and varied responses based on KRAS or EGFR mutational status, reinforcing the importance of personalized medicine approaches in the treatment of cancer. Understanding these dynamics can help refine therapeutic strategies, potentially

leading to the development of more effective second-line treatments or combination therapies that can address resistance or druggability issues in oncogene addiction.

2.2 Oncogene-Addicted Cohorts Gain Sensitivity to Oncogene-Targeted Therapies

The robustness of the OncoStratifier approach was assessed by examining if oncogene addicted cohorts gain sensitivity against drugs that specifically target the oncogene. It was hypothesized that such drugs would predominantly fall into the *stratifying sensitivity* (*SS*) and *full sensitivity* (*FS*) groups, which denote a gain in sensitivity when the oncogene is mutated. Confirming this hypothesis, our analysis showed that 44.1% of drugs targeting the tested oncogenes were categorized as *SS*, and 22.5% as *FS*. In contrast, fewer drugs were categorized under *stratifying resistance* (*SR*) at 14.4%, and *full resistance* (*FR*) at 18.9%, with the latter category showing minimal responsive cell lines in oncogene addicted cohort (**Error! Reference source not found.a**).

The drugs classified under *SR* and *FR* can be attributed to two main factors. First, the influence of secondary targets of the drugs might confound their efficacy. Secondly, the effects of the oncogene may vary by cancer type. A case in point involves drugs targeting EGFR, which is a crucial oncogene in glioblastoma(26), non-small cell lung(27), head and neck(28), colorectal(17), and pancreatic cancers(29). In these cancer types, we found 4 significant drugs in the *SS* category and 3 in *FS*, signifying a gain in treatment sensitivity. However, only 2 drugs, *Pelitinib* and *CUDC-101*, were identified in the *SR* category with score less than 0.4, both in non-small cell lung cancer, where nearly half of the oncogene-addicted cohort still responded to both drugs, highlighting the complex dynamics of drug response. Moreover, *CUDC-101* also targets HAC1-10 and ERBB2 genes as well as EGFR, thus it's low selectivity makes *CUDC-101* less reliable.

Further analysis was performed on oncogene-targeting drugs within each cancer type, focusing only on types where at least 10 significant drugs were found in any category to ensure a valid analysis. Results indicated notable sensitivity gains in breast cancer (51.72% *SS*, 44.83% *FS*), colorectal carcinoma (57.69% *SS*, 19.23% *FS*), ovarian cancer (80% *SS*, 20% *FS*), melanoma (30.77% *SS*, 38.46% *FS*), B-Cell Non-Hodgkin's Lymphoma (45.45% *SS*, 27.27% *FS*), and gastric carcinoma (53.85% *SS*, 23.08% *FS*). Conversely, in non-small (16.67% *SS*, 25% *FS*) and small cell lung carcinomas (36.84% *SS*, 10.53% *FS*), the drugs identified did not predominantly fall into categories that

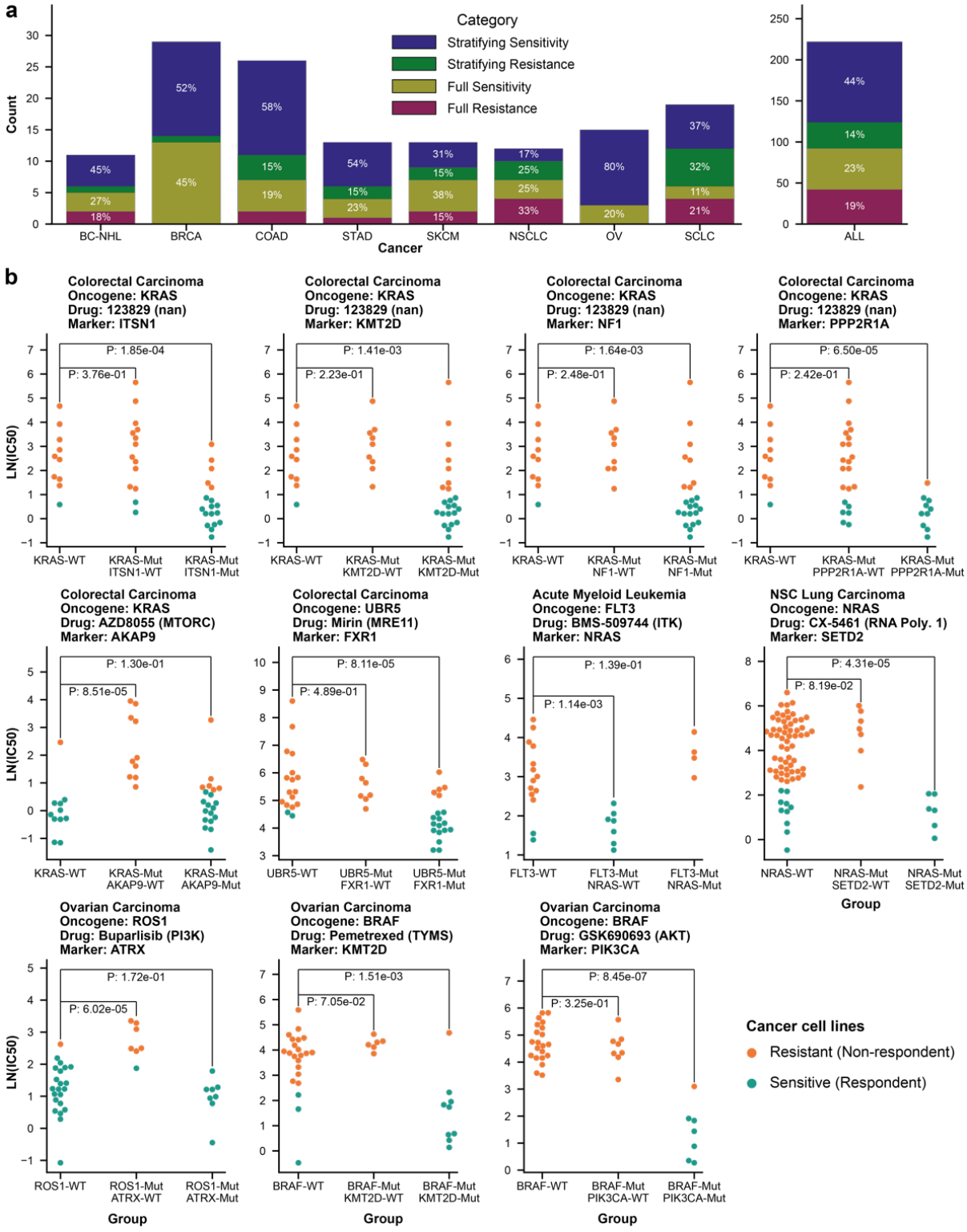

Figure 2: Distribution of oncogene targeting significant drugs and mutational markers of oncostratifiers for well-known oncogene addictions a, Distribution of significant drugs found in an oncogene addiction where the oncogene is also targeted by the drug. **b,** Response of oncogene-WT cohort as well as oncogene addicted cohorts with and without mutation in the mutational markers of oncostratifiers found for well-known oncogene addictions. P values refer to significance of change between oncogene-WT cohort and other cohorts using Fisher-s exact test with BH correction.

indicate sensitivity gain, underscoring the variable efficacy of oncogene-targeting drugs across different

tumor types.

2.3 Drugs Repeatedly Impacted by Oncogene Addiction

Oncogene addiction influences tumor development through complex interactions involving multiple genes and pathways. This dependency often modulates the effectiveness of drugs targeting various molecular pathways. Our OncoStratifier framework identified several drugs with stratifying characteristics frequently observed across multiple cancer types and oncogenes.

Specifically, seven drugs were repeatedly categorized as stratifying sensitivity (*SS*) in over 100 distinct oncogene-cancer pairs **(Fig S1)**. These drugs include CX-5461, Oxaliplatin, Cisplatin, 5-Fluorouracil, Mirin, Afatinib, and Methotrexate, with Gemcitabine also consistently appearing in the *SS* category. Notably, CX-5461(30,31), Oxaliplatin(32–34), Cisplatin (35–37), Methotrexate(38), and Gemcitabine (39) primarily interfere with RNA and/or DNA synthesis and cause DNA damage. In contrast, Mirin prevents homology-dependent repair by affecting G2/M checkpoint(40), Afatinib targets tumor growth factors which are important in multiple cancer types(41–44), and 5-Fluorouracil (45,46) impairs the synthesis pyrimidine which then induces apoptosis. These mechanisms suggest a broader impact on cellular processes crucial in oncogene-addicted cells, reflecting why disruptions in nucleic acid metabolism are particularly effective. Morever, these inhibitors may need specific requirements for their effectiveness, such as Cisplatin needing ERK activation to induce apoptosis (37) or knockdown of NFBD1 and MDC1 enhancing the impact of cisplatin and 5-fluorouracil (46) which further support the change the response against these drugs with oncogene addiction.

The pervasive effectiveness of these drugs across various contexts is also underscored by their frequent use alone or in combination therapies in multiple cancer types. The link between oncogenic addiction and enhanced sensitivity to drugs affecting DNA/RNA synthesis implies that oncogene-addicted cells may rely more heavily on these fundamental processes, making them more vulnerable to such interventions.

Further investigation into the specific pathways and oncogene interactions with these drugs could provide deeper insights into the mechanisms by which oncogene addiction alters drug sensitivity. Additionally, exploring patterns of resistance development and the efficacy of combination therapies involving these drugs could inform more effective treatment strategies for oncogene-addicted cancers.

2.4 Identifying Mutational Markers for Stratifying Drugs

In the pursuit of transitioning from in vitro cell line data to in vivo patient tumors or PDX models, it is crucial to identify biological markers that can segregate similar drug responses for the stratifying drugs that the Oncostratifier identified. Thus, we investigated genetic markers such as mutational status that correlate with drug stratification in oncogene-addicted cohorts.

We identified 59 mutational markers associated with 36 stratifying drugs across 36 distinct genes and 11 cancer types, encompassing 46 cancer type-oncogene pairings (**Error! Reference source not found.b**, **Sup. Fig. S2, S3, S4, S5**). For 7 of these pairings, there was established literature support linking the oncogene to cancer, reinforcing the relevance of our findings (Supplementary Fig.).

Acute Myeloid Leukemia (AML) and FLT3 Addiction (47)**:** We identified *BMS509744* as a stratifying drug (*SS*) with *NRAS* as a mutational marker in the context of FLT3 oncogene addiction in AML. Within the oncogene-addicted cohort, only the subcohort with NRAS WT exhibits gained sensitivity to the ITK inhibiting drug *BMS509744* ($P \approx 1.14 \times 10^{-3}$), while the NRAS mutated subcohort remained resistant ($P \approx 1.39 \times 10^{-1}$). This delineation underscores the pivotal role of NRAS status in modulating response to *BMS-509744* and highlights the drug's potential in targeting FLT3-addicted leukemia with specific genetic backgrounds.

Colorectal Cancer: For colorectal cancer characterized by KRAS addiction, two stratifying drugs were highlighted: *123829* and *AZD8055*. The drug *123829*, categorized as *SS* (stratifying sensitivity), is associated with four mutational markers: ITSN1, KMT2D, NF1, and PPP2R1A. Notably, only the cell lines exhibiting mutations in KMT2D and/or NF1 gain sensitivity to *123829* with KRAS addiction ($P \approx 1.41 \times 10^{-3}$ and $\approx 1.64 \times 10^{-3}$, respectively). Conversely, cell lines with wild-type KMT2D and/or NF1 stay predominantly resistant to *123829*. Interestingly, almost all cell lines with PPP2R1A mutations demonstrate sensitivity to *123829* ($P \approx 6.50 \times 10^{-5}$), suggesting that PPP2R1A mutation status can be a robust predictor of sensitivity to this drug.

On the other hand, the drug *AZD8055*, which falls under the *SR* (stratifying resistance) category, shows a unique pattern of association with only one mutational marker, AKAP9. In KRAS-addicted colorectal cancer cell lines, those with the wildtype AKAP9 gene exhibit resistance to *AZD8055*, indicative of a loss in drug sensitivity correlated with KRAS addiction ($P \approx 8.51 \times 10^{-5}$). However, in cell

lines where AKAP9 is mutated, more than half remain sensitive to *AZD8055*, demonstrating that AKAP9 mutations may mitigate the loss of sensitivity usually associated with KRAS addiction.

Furthermore, we have identified the *Mirin* as a stratifying drug with FXR1 mutation status as a marker in UBR5-addicted colorectal cancer cell lines. UBR5 is involved in damage response and apoptosis (48) and also found to be potentially an oncogene in colorectal carcinoma (49). With the UBR oncogene addiction, only the cell lines with FXR1 mutation gain sensitivity to *Mirin* significantly ($P \approx 8.11 \times 10^{-5}$) compared to UBR WT cohort. Thus, FXR1 can be used as a marker to decide which UBR5 addicted colorectal carcinoma patients should be given *Mirin* as a treatment.

Non-Small Cell Lung Cancer (NSCLC): In NSCLC, *CX-5461*, targeting RNA polymerase I, was effective in NRAS-addicted cohorts with SETD2 mutations ($P \approx 4.31 \times 10^{-5}$), suggesting a potential synthetic lethal interaction between NRAS and SETD2 in the presence of CX-5461 drug.

Ovarian Cancer: In ovarian cancer, Buparlisib (targeting ROS1 addiction), Pemetrexed (targeting BRAF addiction), and GSK690693 (targeting BRAF addiction) showed differential effectiveness in oncogene-addicted cohorts based on ATRX, KMT2D, and PIK3CA mutations, respectively. The effectiveness of Buparlisib was compromised in ROS1-mutated cohorts with WT ATRX ($P \approx 6.02 \times 10^{-5}$), whereas Pemetrexed and GSK690693 showed increased sensitivity in BRAF-addicted cohorts with mutated KMT2D ($P \approx 1.51 \times 10^{-3}$) and mutated PIK3CA ($P \approx 8.45 \times 10^{-7}$), respectively, illustrating the potential for KMT2D and PIK3CA as a stratifying marker in BRAF-driven ovarian cancers.

These findings underscore the complexity of drug responses in oncogene-addicted cohorts and highlight the importance of genetic markers in predicting therapeutic outcomes. Identifying such markers not only aids in understanding drug mechanisms but also assists in tailoring personalized treatment strategies for cancer patients.

2.5 Stratification of Oncogene-Addicted Patients Based on Mutational Markers

We utilized the TCGA patient cohort to stratify oncogene-addicted patients according to the mutational status of previously identified markers. For each cancer type-oncogene-mutational marker pairing, we assessed whether there were sufficient numbers of patients exhibiting oncogene addiction, and if the stratification by mutational marker resulted in adequately sized subcohorts (at least three patients in both WT and mutated groups) to potentially apply targeted therapy (**Error! Reference source not found.**).

Table 1: **Number of oncogene addicted TCGA patients (per cancer type-oncogene pair) stratified by the mutational marker, and differentially expressed genes(DEGs).** The drug response in bold subcohorts is significantly different (P < 0.05) compared to cohorts without oncogene addiction in cancer cell lines. * sign before the value shows more resistance and * sign after the value refers to a better response.

Cancer	Oncogene	Marker	Oncogene Addicted Patients	Marker Mut	Marker WT	DEGs	Tested Genes
AML	FLT3	NRAS	52	2	**50***	12	16758
BRCA	BRD4	PIK3R1	8	**3***	5	40	19170
BRCA	MSI2	NR4A2	5	**1***	4	120	19004
BRCA	ROS1	CHD9	22	**6***	16	79	19556
COAD	JAK3	MAP2K1	17	3	**14***	170	19121
COAD	KRAS	AKAP9	220	16	***204**	1140	19943
COAD	KRAS	ITSN1	220	**14***	206	70	19943
COAD	KRAS	KMT2D	220	**29***	191	2028	19943
COAD	KRAS	NF1	220	**8***	212	14	19943
COAD	KRAS	PPP2R1A	220	**6***	214	93	19943
COAD	KSR2	FXR1	25	**3***	22	17	19263
COAD	MECOM	FXR1	36	**7***	29	7	19362
COAD	MTOR	FXR1	43	**7***	36	5	19425
COAD	MYBL1	MAP2K1	20	4	**16***	17	19201
COAD	NTRK3	FXR1	24	**5***	19	14	19149
COAD	NUP98	CNOT1	26	13	**13***	79	19241
COAD	NUP98	PIK3CA	26	11	**15***	20	19241
COAD	NUP98	PTEN	26	7	**19***	30	19241
COAD	UBR5	FXR1	33	**4***	29	25	19408
COAD	WWP1	FXR1	27	**6***	***21**	17	19300
COAD	WWP1	PIK3CA	27	**13***	***14**	79	19300
GBM	IGF1R	EGFR	3	***1**	2	477	18610
NSCLC	KDM5A	ARID1A	13	**2***	11	340	19498
NSCLC	LGR5	CLSPN	24	***1**	23	793	19682
NSCLC	MAP3K13	CLSPN	14	***1**	13	18	19493
NSCLC	MGAM	TP53BP1	58	3	55	4	19863
NSCLC	NTRK3	KEAP1	48	15	**33***	1392	19832
NSCLC	PDGFRB	MAP4K3	17	2	**15***	125	19673
SCLC	CARD11	TJP1	32	**3***	29	18	19719
SCLC	INSR	MED12	14	**1***	13	13	19435
SKCM	JAK3	ANK3	20	14	***6**	38	19722

In the majority of cases, the marker-WT subcohort was larger than the marker-mutated subcohort. This finding is significant because if the WT status of the marker is linked with a better response in oncogene-addicted cell lines compared to non-addicted cohorts, it implies a substantial patient group

could benefit from the stratifying drug. Notable instances include FLT3 addiction in AML, where 96% (50/52) of the patients possess WT NRAS, showing a better response to the drug BMS-509744; JAK3 and MYBL addictions in COAD with 80% (14/17) and 82% (16/20) people having WT MAP2K1, respectively; and NUP98 addiction in COAD where over 50% of the patients have a WT marker (CNOT1, PIK3CA, or PTEN) associated with better responses to *HG558801*.

Conversely, even with smaller numbers, the mutated marker gene often correlates with improved responses, indicating that, with oncogene addiction, sensitivity enhancements are confined to cohorts with the mutated marker. For example, in COAD with KRAS addiction, out of 220, we identified 14 and 29 patients possessing mutated ITSN1 and KMT2D markers, respectively. In another case, 13 WWP1-addicted COAD patients with mutated PIK3CA also showed enhanced drug sensitivity.

Additionally, PIK3CA was identified as a critical mutational marker for response to the drug *Mirin* in WWP1-addicted COAD patients. In this group, patients with mutated PIK3CA demonstrated significantly increased sensitivity to *Mirin*, whereas those with WT PIK3CA lost sensitivity, highlighting the mutation's dual role in modifying drug response. This clear dichotomy makes PIK3CA a valuable predictive marker for therapeutic strategies in WWP1-addicted COAD, with nearly equal division of patients into mutated and WT subcohorts.

2.5.1 Differentially expressed genes within oncogenic addicted cohort and their enrichment

We further investigated the oncogene-addicted patient cohorts and their stratification by the mutational marker to identify what could cause the change in response between two groups that are split by the mutational marker. For this purpose, we first found differentially expressed genes (DEGs) between subcohorts that are split by mutational markers (**Error! Reference source not found.**), then we also analyzed these DEGs and conducted an enrichment analysis for these DEGs. We limited our investigation to cases where both cohorts with the mutated marker and the WT marker have at least 10 patients.

We identified 220 colorectal patients who are addicted to the KRAS oncogene. Previously, we identified that significant loss in sensitivity against drug AZD8055 with KRAS addiction only within AKAP9 WT cohort which includes 204 of 220 patients. Thus, AKAP9 would be an efficient marker to eliminate KRAS

addicted patients who would not benefit from an mTORC targeting drug. Furthermore, we identified 1140 DEGs between AKAP9 mutated and WT KRAS addicted cohort where these DEGs were enriched mostly in major histocompatibility complex (MHC) related gene ontology terms as well as interferon gamma response hallmark which is related to apoptosis and cycle. The deterioration in MHC class 1 molecules are already known to cause ineffectiveness in some cancer treatments (50). Thus, the difference in response in both cohorts can be cause due to KRAS addiction impacting the MHC class in some patients. Similarly, Interferon-gamma (IFN-γ) plays a vital role in boosting the immune system's capacity to identify and destroy cancer cells (51), making it essential for the effectiveness of certain therapies (52).

Another marker was KMT2D whose mutated version was associated with sensitivity gain against the drug 123829 with KRAS addiction in colorectal carcinoma. 29 of 220 patients in oncogene addicted patients had KMT2D mutation which could benefit the treatment with 123829. Further analysis with KRAS addicted patients showed that 2028 genes are differently expressed between KMT2D mutated and WT cohorts. Aligned with previous results, these genes were also enriched MHC related GO terms and Interferon Gamma Response hallmark.

2.5.2 Validating KRAS-Oncogene Stratifying Drugs on PDX samples.

TBW…

2.6 Potential Application: Drug Sets to Cover Oncogene Addicted Cohort

Although stratifying oncogene addiction patients is crucial to define subcohorts that might be sensitive to training due to the impact of oncogene mutation, there will be still one subcohort sensitive to the drug and one subcohort resistant to it. Thus, not all oncogene-addicted patients are treatable by a stratifying drug that is found. To bring the potential treatment option to all of the oncogene-addicted cohorts, we investigated stratifying drugs that can cover the whole set of oncogene-addicted cell lines (**Supplementary Table S3**).

We identified 62 cases in various cancer types where there are at least 10 oncogene addicted cell lines and all of them can be covered by only 2 stratifying drugs. Out of these 62 cases, 3 of them were oncogene cancer type pairs which is supported by the literature.

ERBB2 or widely known as “HER2” is strongly associated with poor prognosis in breast cancer (53,54) and impacts the cell growth, differentiation, and migration together with the other members of EGFR (55). ERBB2 addicted cohort of 12 cell lines in breast cancer was covered by Afatinib (9 CCLs) and Telomerase Inhibitor IX (8 CCLs). The drug *Afatinib* is already used to target ERBB2 (56); however, to the ERBB2 addicted cohort that is resistant to *Afatinib* (57), the second drug *Telomerase Inhibitor IX* can be an opportunity.

MDM2 is an oncogene due to highly expressed MDM2 supressing TP53 which increases the risk of cancer (58). Inhibition of MDM2 selectively targets PTEN-deficient CRC cells, activating p53 and inhibiting tumor growth (59). MDM2 addicted colorectal cancer cohort (11 CCLs) was covered by HDAC1 targeting *AR-42* (8 CCLs) and AURKA targerting *Alisertib* (6 CCLs) drugs. HDAC1 can deacetylate p53 by binding to MDM2 (60) and AURKA enhances the p53 degradation effect of MDM2 (61), thus; inhibiting both genes can potentially reduce the p53 degradation and could lead to better prognostic outcome in different patients that are MDM2 mutated.

Moreover, UBR5 is an oncogene associated with poor prognosis in gastric carcinoma (62). Oncostratifier showed that UBR5 addicted gastric cancer cohort (12 CCLs) can be covered by PIK3CG targeting *PIK-93* (8 CCLs) and FEN1 targeting *FEN1* (6 CCLs) drugs.

Furthermore, we found cases where 1 or 2 cell lines in the oncogene addicted cohort are never sensitive to any of the stratifying drugs we have identified (**Supplementary Table S4**). Thus, we also investigated the set cover drugs with cell lines that are sensitive to at least 1 stratifying drug. Doing this, we were able to identify drug set covers with only 2 stratifying drugs also for other well-known oncogene addictions such as FLT3 addiction in Acute Myeloid Leukemia (AML); EGFR addiction in Glioblastoma; AKT1, DDR2, MET, NRAS, PIK3CB, RET, and ROS1 addiction in non-small cell lung carcinoma (NSCLC); and EGFR and MET in small cell lung carcinoma.

3 Conclusion

In this study, we proposed Oncostratifier, a statistical framework that leverages drug response data on cancer cell lines to find stratification opportunuties for oncogene addicted cohorts in cancer. Although, previously stratification methods are used extensively for cancer patients, to our knowledge, Oncostratifier is the first method to stratify oncogene addicted cohorts computationally and

systematically. The nature of the stratifying oncogene addicted cohort is a more complex problem compared to stratifying cancer patients as whole since the latter has the advantage of abundant data and usually annotations to support the stratification. Moreover, our systematical approach depends on existing drugs, thus making our results directly usable for clinical trials.

Oncostratifier identified stratifying drugs and stratification on cohorts for multiple oncogene addictions in different cancer types including well-known and researched addictions such as EGFR, KRAS, and UBR5 addictions in colorectal carcinoma, MYC addiction ovarian carcinoma, and ERBB2 addiction in breast cancer. Furthermore, some of these oncogene addictions are known to be untargetable such as KRAS in colorectal carcinoma or having resistance such as EGFR in colorectal carcinoma. Thus, making our finding more valuable.

For this problem, there is no ground truth or annotation that can support our findings. By making use of the fact that oncostratifier also recognizes sensitivity gain and loss on drugs with oncogene addiction, we validated our approach and findings. Oncostratifier showed that drugs, which target a specific oncogene and also stratify oncogene addicted cohorts of that specific oncogene where the drug targets that specific oncogene, are more likely to be in the oncostratier categories that shows sensitivity gain.

To make a transition from cancer cell lines to patients, we also identified mutational markers for stratifying drugs found by oncostratifier. These markers can stratify patients similar to stratifying drugs, thus can be used in cases where there is a lack of drug response data, such as TCGA patient data. We also showed that with mutational markers found, we can also stratify oncogene addicted patients for more specific treatments.

PDX related part….

Oncostratifier also uncovered a treatment opportunity for oncogene addicted cohorts. For some oncogene addictions, using multiple stratifying drugs that are identified for a specific oncogene addiction, all of the oncogene addicted cohorts can be treated by using 2 or more of these stratifying drugs.

4 Methods

4.1 Data collection and processing

4.1.1 Cancer cell lines

We systematically compiled a comprehensive dataset of cancer cell lines, complete with annotations and a detailed list of mutations (version 20220510), sourced from the Cell Model Passports (63). The mutation status for each gene was determined by focusing exclusively on non-silent variants to ensure relevance and specificity in our analysis.

4.1.2 Drug response

Our study harnessed drug response data for cancer cell lines from the Genomics of Drug Sensitivity in Cancer (GDSC) database (64,65) (version 27Oct23). This dataset encompasses a broad spectrum of compounds, 367 for GDSC1 and 198 for GDSC2, each with their putative targets. In instances of overlap between GDSC1 and GDSC2, drugs were treated as distinct drugs to preserve the integrity of our analysis. The drug efficacy against specific cancer cell lines was quantified using the natural logarithm of the 50% growth inhibition values (*ln(IC50)*). To further our understanding, we transformed these *ln(IC50)* response scores into binary responses, employing the natural logarithm of the peak plasma concentration (*ln(CMax)*) as a threshold. This transformation facilitated the categorization of cell line responses into either sensitive (responder) if *ln(IC50)* is less than *ln(CMax)* or resistant (non-responder) groups if *ln(IC50)* is greater than *ln(CMax)*.

4.1.3 Oncogenes

The oncogenes to be tested were obtained from MSK's Precision Oncology Knowledge Base (66) (latest version as of 10/02/2023). Our stringent selection criteria considered only those genes recognized as oncogenes in at least one tissue ("Is Oncogene" == "Yes") and not classified as tumor suppressor genes in any tissue ("Is Tumor Suppressor Gene" == "No").

4.1.4 Primary tumours

We gathered an array of gene expression, mutation, and clinical data for primary tumor samples from the TCGA patient tissue samples, courtesy of the Pan-Cancer Atlas (13). The processed and curated data was accessed through the cBio portal (67,68) (accessed on 15/12/2023). Our analysis considered only primary tumors as samples. In addition, we remove silent mutations from the mutation data. For

differential gene expression analysis, we leveraged RSEM (Batch normalized from Illumina HiSeq RNASeqV2) mRNA expression data. Moreover, to visualize the normalized expression values of differentially expressed genes, we employed log-transformed mRNA expression (RNA Seq V2 RSEM) z-scores compared to the expression distribution of all samples.

4.2 Oncostratifier

4.2.1 Finding & categorizing stratifying drugs

Our approach started with the stratification of cancer cell lines into two distinct cohorts: $Oncogene_{Mut}$ (mutated) and $Oncogene_{WT}$ (wild-type), based on the mutational landscape of the relevant oncogene within each cell line. This stratification was restricted by a criterion of minimum representation: for an oncogene to be included in our analysis, both its mutated and wild-type (WT) variants had to be represented by at least three distinct cell lines to reduce the total amount of tests and ensure a robust analysis. The variability (uncertainty) in drug response within these cohorts was analyzed using Shannon entropy, a metric that encapsulates the uncertainty inherent in the drug response profiles of each group:

$$H\,(Oncogene_{Mut}) = -p_S \log_2 (p_S) - p_R \log_2 (p_R)$$

$$H\,(Oncogene_{WT}) = -q_S \log_2 (q_S) - q_R \log_2 (q_R)$$

where p_S and p_R (q_S and q_R) represent the proportions of sensitive and resistant cancer cell lines within the $Oncogene_{Mut}$ ($Oncogene_{WT}$) cohort, respectively. To discern drugs that selectively stratify one cohort but not the other, we examined the differential entropy (ΔH) between these cohorts, calculated as:

$$\Delta H = H\,(Oncogene_{Mut}) - H\,(Oncogene_{WT})$$

A positive ΔH value indicates drugs that exhibit pronounced stratification in the $Oncogene_{Mut}$ cohort with a minimal impact on the $Oncogene_{WT}$ cohort. Conversely, a negative ΔH underscores drugs that predominantly stratify the $Oncogene_{WT}$ cohort. To ascertain the statistical significance of these ΔH values, we conducted a permutation test with 10,000 iterations. Each iteration involved the random assignment of response labels to the cancer cell lines and the computation of the ΔH score. This rigorous process enabled us to derive p-values to assess the probability of getting an extreme value than the actual observed ΔH, when the null hypothesis, positing no discernible difference in entropy between the two cohorts, is true:

$$p - value = \frac{\text{Number of iterations where } |\Delta H_{random}| \geq |\Delta H_{observed}|}{\text{Total number of iterations } (10{,}000)}$$

According to the entropy values of each cohort and ΔH, drugs with a significant entropy score (*p-value*< 0.05) were categorized as follows:

- **Stratifying sensitivity (*SS*):** $\Delta H > 0$; p−value < 0.05; $q_S < q_R$
- **Stratifying resistance (*SR*):** $\Delta H > 0$; p−value < 0.05; $q_S > q_R$
- **Full sensitivity (*FS*):** $\Delta H < 0$; p−value < 0.05; $p_S > p_R$
- **Full resistance (*FR*):** $\Delta H < 0$; p−value < 0.05; $p_S < p_R$

The stratifying categories denote cancer cell lines acquiring stratification potency through either the acquisition (*SS*) or loss (*SR*) of sensitivity in different subcohorts due to oncogenic addition. Although the cell lines are stratified in the $Oncogene_{Mut}$ both for *SS* and *SR*, their difference arises from the drug response in the $Oncogene_{WT}$ where the cell lines are either dominantly resistant (*SS*) or sensitive (*SR*). Conversely, other groups signify a reduction in stratification capability in the $Oncogene_{Mut}$ group due to the resistant cell lines gaining (*FS*) or sensitive cell lines losing (*FR*) sensitivity.

4.3 Further Analysis of Stratifying Drugs

4.3.1 Finding mutational markers for stratifying drugs

We revealed markers for drugs that stratify oncogene-mutated cell lines by an association analysis between drug response (sensitive or resistant) and the mutational status of each cancer-related gene in both the $Oncogene_{Mut}$ and $Oncogene_{WT}$ cohorts. This analysis employed Fisher's exact test, focusing on genes previously identified as cancer genes (CGs) amid cancer functional events (CFEs) (65). Due to the multiplicity of genes tested for each stratifying drug, the p-values from Fisher's exact test were corrected using the Benjamini-Hochberg (69) procedure. A gene was deemed a mutational marker of a stratifying drug if the corrected p-value in the $Oncogene_{Mut}$ cohort was below 0.05, but not in the $Oncogene_{WT}$ cohort.

4.3.2 Differential gene expression analysis

To transition our investigation to the primary tumor level, considering only the primary tumor samples where the oncogene is mutated, we discerned differentially expressed genes between subcohorts where the marker gene was either mutated or wild-type (WT). Initial steps involved the exclusion of

genes with variance below 0.0001 in the rounded RSEM read counts. Counts that turned negative due to

RSEM adjustments were reset to 0. The processed matrix was then inputted to the DeSeq2 (70) algorithm (pyDeSeq2 (71)). Notably, while DeSeq2 necessitates raw read counts as input, it also allows the use of rounded RSEM read counts (72). DeSeq2 was executed twice: initially to identify Cook outliers, and subsequently to identify differentially expressed genes after imputing values filtered out based on Cook's distance. As suggested by DeSeq2, only the genes whose p-values passed the independent filtering stage (70) were identified and then adjusted via the Benjamini-Hochberg procedure (69). Lastly, genes with adjusted p-values below 0.05 were classified as differentially expressed.

4.3.3 Gene enrichment analysis

We investigated whether the differentially expressed genes associated with each cancer-oncogene-marker pair were significantly enriched within various gene sets, drawing from Gene Ontology (version 2023; encompassing biological processes, cellular components, and molecular functions) (73), MSigDB Hallmark (74), and canonical pathways from databases such as KEGG (2021, Human) (75), WikiPathway (2023, Human) (76), and Reactome (2022) (77). This analysis was facilitated by EnrichR (version June 8, 2023, accessed on 17/01/2023) (78), which employs Fisher's exact test to evaluate enrichment and utilizes the Benjamini-Hochberg procedure (69) to adjust p-values, accounting for multiple testing.

5 Supplementary Information

Supplementary Tables:

- **Table S1:** Detailed results of every drug found significant over all cancer type-oncogene pairs. The table includes the found drug categories, entropy scores in both cohorts, oncostratifier score (ΔH), oncostratifier p-value, mutational marker if found, and additional statistics on oncogene addicted patients for each case as well as differentially expressed genes for the mutational markers found.

- **Table S2:** Number of oncostratifier-drug and oncogene pairs found for each cancer type and their assigned categories.
- **Table S3:** For each cancer type-oncgene pair, set of stratifying drugs that can cover all oncogene addicted cancer cell lines.
- **Table S4:** For each cancer type-oncgene pair, set of stratifying drugs that can cover all oncogene addicted cancer cell lines where each cancer cell line has at least 1 responsive stratifying drug.

Supplementary Document: Includes supplementary methods, results and figures.

Acknowledgements

TBD…

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