The universe of normal and cancer cell line responses to anticancer treatment: Lessons for cancer therapy

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Abstract

According to the Surveillance Epidemiology and End Results report, 1,479,350 men and women will be diagnosed with and 562,340 will die of cancer of all sites in 2009, indicating that about 40% of the cancer patients do not respond well to current anticancer therapies. Using tumor and normal tissue cell lines as a model, we show this high mortality rate is rooted in inherent features of anticancer treatments. We obtain that, while in average anticancer treatments exhibit a two fold higher efficacy when applied to cancer cells, the response distribution of cancer and normal cells significantly overlap. Focusing on specific treatments, we provide evidence indicating that the therapeutic index is proportional to the fraction of cancer cell lines manifesting significantly good responses, and propose the latter as a quantity to identify compounds with best potential for anticancer therapy. We conclude that there is no single treatment targeting all cancer cell lines at a non-toxic dose. However, there are effective treatments for specific cancer cell lines, which, when used in a personalized manner or applied in combination, can target all cancer cell lines.

Background

Cell culture studies are the starting point of most screens for anticancer treatments [1, 2]. They provide an initial idea of their anticancer efficacy, when applied to tumor derived cell lines, and toxicity, when applied to cell lines derived from normal tissue. Thus, high throughput cell based assays has been used to identify novel anticancer drugs. One of the largest screens, as measured by the number of treatments tested, is the NCI60 screen, the anticancer drug screen run by the National Cancer Institute (USA) [1]. Specifically, the NCI60 screen has reported the growth inhibition efficacy of about 50,000 compounds against 60 tumor derived cell lines, of different tissue of origin and harboring different somatic alterations. This screening effort has lead to the identification and characterization of compounds with potential anticancer activity {Shoemaker, 2006 #674}, including small molecules selectively toxic in P-glycoprotein expressing tumor cells [3] and compounds with increased activity in tumor cells carrying the BRAF-V600E mutation [4].

More recently, the Genomics of Drug Sensitivity in Cancer Project has being launched as part of a collaboration between The Cancer Genome Project at the Wellcome Trust Sanger Institute (UK) and the Center for Molecular Therapeutics of the Massachusetts General Hospital Center (USA). This project has been designed to screen potential anticancer drugs against a larger library of tumor derived cell lines, at expenses of focusing on a smaller subset of compounds. Examples of studies carry on through this research initiative include the screening of lung cancer cell lines for sensitivity to an EGFR inhibitor [5] and of the sensitivity of cancer cell lines to kinase inhibitors [6].

Yet, because these screening efforts focus on the most potent treatments, there is hardly any analysis of the overall response of cancer and normal cell lines to anticancer treatments. Such analysis is imperative to have a more complete understanding of the screening data and to statistically differentiate a good response from the average response. Furthermore, given that most anticancer screen are based on panels of tumor derived cell lines, it is not clear whether we can make any conclusion about toxicity based on them. Here, we provide a first attempt to answer those questions based on a recollection of literature data reporting growth

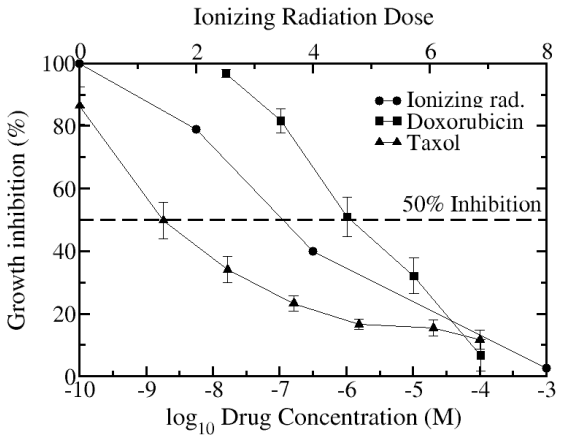

Figure 1 **Growth inhibition assay:** The drug response curves of the MCF7 breast cancer cell line to ionizing radiation, as a function of radiation dose (top scale), and to the chemotherapeutic compounds Doxorubicin and Taxol, as a function of their $\log_{10}$ concentrations (bottom scale). The symbols represent the average and the error bars the corresponding standard error.

inhibition measurements and the NCI60 and Sanger screen data.

Results and Discussion

In order to compare the efficacy of different treatment types (e.g., radiation vs small molecule) and different range of activity for the same treatment type (e.g., two small molecules with different concentration ranges), we first need to identify the appropriate quantity to compare them (Fig. 1). For example, growth inhibition assays manifest similar features for ionizing radiation and small molecule treatments, but only when using a linear radiation dose and a logarithmic concentration scale, respectively. Within small molecules treatments, two compounds can manifest 50% growth inhibition at different concentration ranges, such as nM for Taxol and μM for Doxorubicin (Fig. 1). To be more precise let us focus on the 50% growth inhibition (Fig. 1, dashed line). In the case of radiation, this is achieved at the ID50, the radiation dose resulting in the 50% growth inhibition of treated cells relative to untreated controls. In the case of small molecule treatment, this is achieved at the IC50, the small molecule concentration resulting in the 50% growth inhibition of treated cells relative to untreated controls. We define the relative dose of treatment T resulting in a 50% growth inhibition of cell line C, IR50(T,C), as IR50(T,C)=ID50(T,C)-mean(ID50)(T) for radiation treatment and IR50(T,C)=$\log_{10}$IC50(T,C)-mean($\log_{10}$IC50)(T) for small molecules, where the mean is taken over the available universe of cell lines. This quantity satisfies the desired properties, independence of treatment kind and range of activity, and it allow us to investigate the overall response statistics to anticancer treatments.

We have collected literature data reporting radiation ID50s and IC50s of several small molecules and antibodies against cancer and normal cell lines (Supplementary Table 1). In total, it comprises 101 different treatments represented by measurements in 4 or more cell lines. The IR50 distribution across the literature dataset exhibits a symmetric distribution with a peak around zero (Fig. 2a). This distribution has a similar shape for both cancer and normal cell lines, with the latter shifted to the right. In average normal cell lines show a two fold higher IR50 than cancer cell lines (p=5x10^{-5}, permutation test), supporting the general expectation that cancer cell lines are more sensitive to radiation and chemotherapy treatment than normal cells. However, there is a significant fraction of normal cell measurements with negative IR50, indicating that some treatments are causing a 50% growth inhibition of normal cells at relatively low doses. Similarly, we can inspect the statistical features of specific treatments. For methotrexate (MTX) the IR50 distribution seems bimodal and it has a more evident separation between the cancer and normal distributions (Fig. 2b). The latter observation is quantified by the, here defined, *Cell Proliferation Therapeutic Index*, CPTI(T)=(μ_N(T)- μ_C(T)/σ (T), where μ_N(T) and μ_C(T) are the mean IR50 of treatment T in normal and cancer cell lines, respectively, and σ(T) is the IR50 standard deviation of treatment T across all cell lines. This quantity characterizes how well the treatment is targeting cancer cell lines compared to normal cell lines, relative to its overall variation across all cell lines. Treatments having large positive values of CPTI are those with the best therapeutic potential. In the case of MTX we obtain CPTI(MTX)=1.2, implying that the average IR50 for cancer cells is one standard deviation above that for normal cells

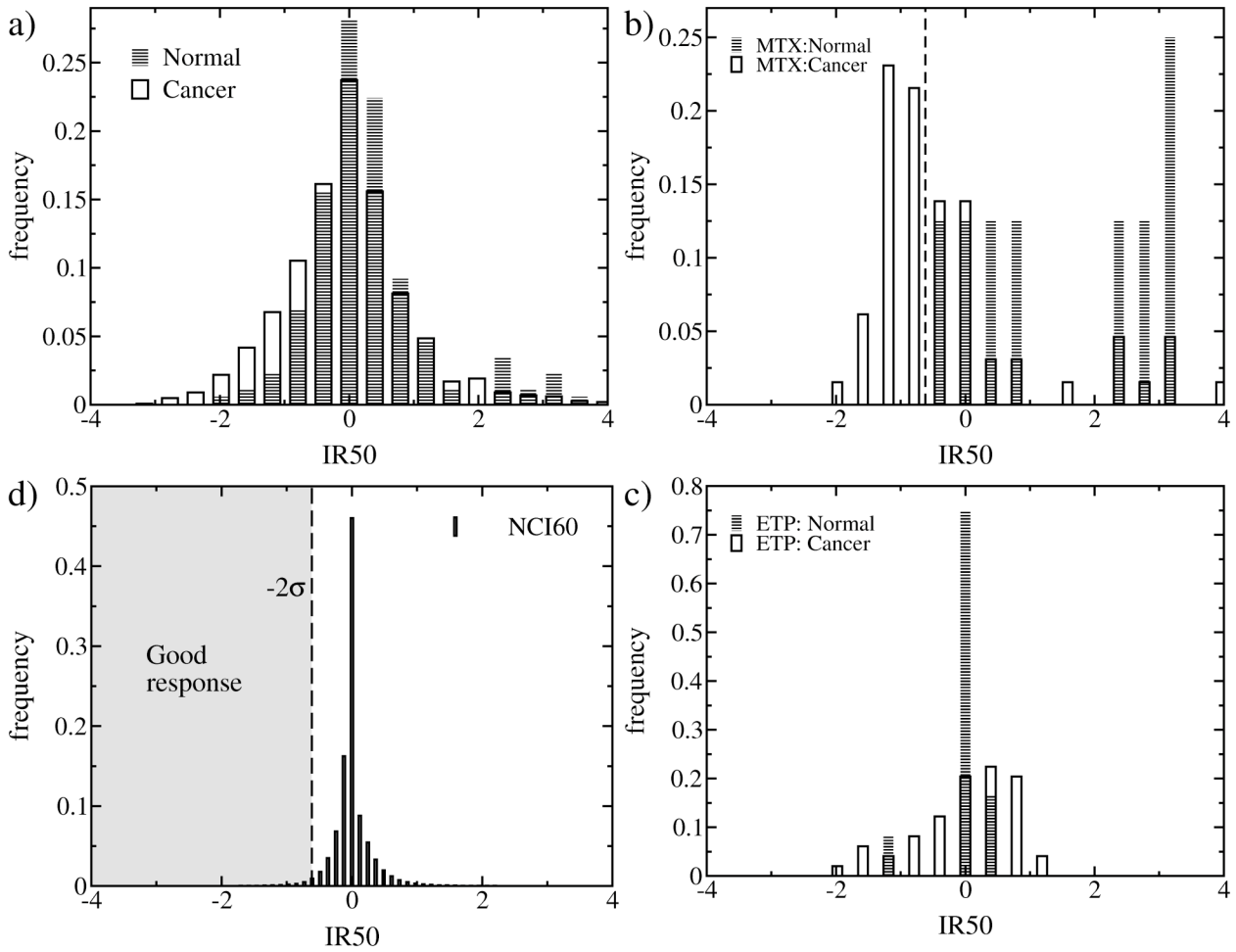

Figure 2 Response distribution: a) The IR50 distribution across several compounds and normal and cancer cell lines as obtained from the literature dataset. The IR50 for specific treatments, b) methrotexate (MTX) and c) etoposide (ETP), is also shown. d) The IR50 distribution across the NCI60 screen. The dashed line at -2 standard deviations splits the IR50s into good responses to the left and not good responses to the right.

(p=0.0015). In contrast, the IR50 distribution for etoposide (ETP) is unimodal and centered at zero for both cancer and normal cells (Fig. 2c). This fact is also captured by the therapeutic index just defined, CPTI(ETP)=-0.09, which is small compared to that for MTX. In total we were able to estimate the CPTI for thirteen compounds having four or more cancer cell lines and four or more normal cell lines in the literature dataset (Supplementary Table 1a). In addition to MTX, Magainin G, Betulinic acid, and Vincristine have a CPTI above one (p<0.05). For three other treatments, 5-Fluorouracil, IR, and Doxorubicin, we obtain a CPTI above 0.4, although we cannot reject the possibility that cancer and normal cell lines have the same IR50 distribution (p>0.05). The remaining compounds show negative CPTI and no significant difference between cancer and normal cell lines.

Ideally we would like to extend this kind of analysis to a larger library of treatments. For example, the NCI60 anticancer drug screen reported the response of 47,624 compounds against 60 tumor derived cell lines (NCI60 screen) [1]. While this screen has not assayed normal cell lines, it has the advantage of having tested several compounds against the same cancer cell lines, following the same experimental protocol. The IR50 distribution of the 47,624×60 measurements (Fig. 2d) shows features similar to the literature IR50 distribution (Fig. 2a), with a peak around zero and a fast decrease as we move away from zero. However, the NCI60 distribution has a more pronounced peak at zero and a lesser spread around it. Given that the NCI60 screen has

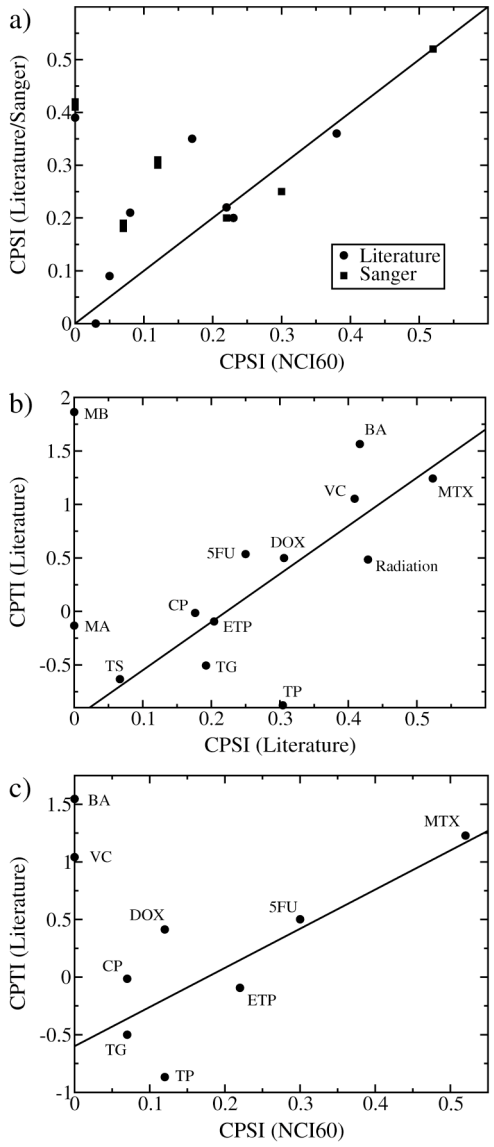

Figure 3 CPSI as a CPTI surrogate: a) Scatter plot of the CPSI estimated from the literature and Sanger datasets as a function of the corresponding value estimated from the NCI60 dataset, each symbol representing a compound. The solid line is the identity and points close to it are indicative of similar results. b) Plot showing a linear correlation between the literature-estimated CPSI and CPTI. The solid line represents the best linear fit to data points with CPSI>0, CPTI=-1.0+4.5CPSI. c) Plot showing a linear correlation between the NCI60-estimated CPSI and the literature-estimated CPTI. The solid line represents the best linear fit to data points with CPSI>0, CPTI=-0.6+3.4CPSI. The following abbreviations have been used, 5-fluorouracil (5FU), butilinic acid (BA), cisplatin (CP), doxorubicin (DOX), etoposide (ETP), methotrexate (MTX), magainin A (MA), magainin B (MB), thioguanine (TG), thiopurine (TP), Thiosemicarbozone NSC73306 (TS), and vincristine (VC).

tested a much larger and less biased treatment library we choose its IR50 distribution as our reference model to identify low IR50 outliers. We define a good response as an IR50 below -2σ (Fig. 2d, dashed line), where $\sigma \approx 0.31$ is the IR50 standard deviation across the 47,624×60 measurements. In simple words, with about 95% confidence, an ID50 dose 0.62 units below the mean and an IC50 two fold lower than the mean is a good response.

A potential limitation of the NCI60 screen is that it does not report data for normal cell lines, forcing us to develop a CPTI surrogate based on measurements for cancer cell lines alone. From the analysis of the literature dataset, we noticed that the left tail of the IR50 distribution is enriched by measurements for cancer cells (Fig. 2a). Thus, we hypothesized a good candidate could be a measurement quantifying the propensity of a given treatment to have measurements in the left tail of the IR50 distribution for cancer cells. Thus, we define the Cell Proliferation Selective Index of treatment T, CPSI(T), as the fraction of cancer cells with IR50 below -2σ standard deviations (about 5% of the extreme low IR50). To investigate the reliability of this quantity we analyzed three datasets with different sample sizes: literature (4 to tens samples), NCI60 (60 samples) and a recent screen by the Sanger Institute (Sanger screen, 355 samples). Overall the CPTI computed from the NCI60 data shows similar values than those obtained using the literature and Sanger measurements (Fig. 3a), with a Pearson correlation coefficient (*PCC*) of 0.34 and statistical significance (*p*, permutation test) of 0.09. This correlation is even stronger (*PCC*=0.76, *p* =0.0035) when excluding CPTIs identical to zero, resulting from treatments with no sample manifesting a good response.

To investigate potential relationships between CPSI and CPTI, we first focused on the literature dataset, where we can estimate both the CPTI and CPSI for 13 treatments (Supplementary Table 2d). After excluding CPTI=0 measurements (2 points), we obtain a remarkably good correlation between the two indexes (*PCC*=0.77, *p*=0.0029) (Fig. 3b). Second, we tested whether the CPSI estimated from the NCI60 screen is predictive of the CPTI from the literature data, using nine treatments for which we had data in both cases (Supplementary Table 2d). Excluding once again

CPSI	CPTI	NSC	Mechanism of action	Name
0.57	1.33	613327	DNA Antimetabolite	Gemcitabine
0.52	1.16	740	RNA/DNA Antimetabolite	Methotrexate
0.48	1.04	733504	mTOR Inhibitor	Everolimus
0.43	0.87	732517	Tyrosine Kinase Inhibitor	Dasatinib
0.42	0.82	683864	mTOR Inhibitor	Temsirolimus
0.40	0.76	712807	RNA/DNA Antimetabolite	Capecitabine, 5FU prodrug
0.38	0.70	226080	mTOR inhibitor	Rapamycin
0.33	0.53	63878	DNA Antimetabolite	Ara-C
0.33	0.53	606869	RNA/DNA Antimetabolite	Clofarabine
0.30	0.42	27640	DNA Antimetabolite	5-Fluorouracil deoxyriboside
0.27	0.31	698037	RNA/DNA Antimetabolite	Alimta
0.25	0.25	287459	DNA Antimetabolite	Cytarabine
0.23	0.19	719344	Aromatase Inhibitor	Anastrozole
0.23	0.19	125066	Alkylating Agent	Bleomycin
0.23	0.19	125973	Antimitotic Agent	Taxol
0.23	0.19	279836	Topoisomerase II Inhibitor	Mitoxantrone
0.23	0.19	266046	Alkylating Agent	Oxaliplatin
0.22	0.14	105014	DNA Antimetabolite	Chorodeoxyadenosine
0.22	0.14	141540	Topoisomerase II Inhibitor	Etoposide
0.18	0.02	127716	DNA Antimetabolite	Decitabine

Table 1 Compounds with highest CPSI: The twenty FDA drugs with the highest CPSI, as obtained from the analysis of the NCI60 data. The CPTI was obtained using the linear relationship CPTI=- 0.6+3.4CPSI derived from Fig. 3c.

the CPTI=0 measurements (2 points), we recapitulated the linear correlation between the CPTI and CPSI (*PCC*=0.80, *p*=0.02) (Fig. 3c). On the other hand, treatments with CPSI=0 and relatively high CPTI are false negatives, resulting in an estimated false negative rate of about 20% (2/9).

The use of the CPSI as a surrogate for the CPTI has important practical implications. Tumor derived cell lines are in generally easier to growth in culture and they are routinely used in anticancer drug screens. In particular, as mentioned above, the NCI60 screen has tested the response of several compounds, including hundred FDA approved drugs (Supplementary Table 1c). Now, armed with the CPSI surrogate of therapeutic index, we can use this dataset to score compounds for their potential use in anticancer therapy. Using the IC50 data reported by the NCI60 screen, we computed the CPSI of 47,624 compounds and, exploiting the linear relationship in Fig. 3c, we made predictions for their CPTI. Table 2 reports the twenty best FDA approved drugs according their predicted CPTI (see also Supplementary Table 2b). Among compounds scoring high, there is a significant enrichment of mTOR inhibitors (*p*=0.05, Fischer's exact test). There are several antimetabolites as well, but it is expected given the abundance of antimetabolites among the hundred FDA drugs analyzed here (*p*=0.21). On the other hand, there is a significant depletion of DNA damaging agents, including alkylating agents (*p*=0.000005), topoisomerase inhibitors (*p*=0.0000007), and antimitotic agents (*p*=0.0006). Taking together these results indicate that mTOR inhibitors and antimetabolites has overall a better predicted CPTI than DNA damaging agents.

A high CPTI is indicative of a good therapeutic potential, because it will allow to set at intermediate treatment dose that would be sufficiently high to target cancer cell lines, but sufficiently low to avoid toxicity over normal cell lines. However, it is extremely important to notice that we still face the problem of certain cancer cell lines being resistant to the treatment. This fact is better illustrated by inspecting the IR50 distribution for a compound with high CPTI, such as MTX. By setting the IR50 at -2σ (Fig. 2b, dashed line) we can guaranty that we are working at a drug concentration below the IC50 for all the normal cell lines considered in this study (non-toxic dose). However, the same

applies for all the cancer cell lines at the right of the dashed line (48%). The traditional solution to this problem has been to increase the dose to cover a higher fraction of cancer cell lines, but at expenses of an increased toxicity. There are, however, other strategies to overcome the problem of resistance without compromising on toxicity.

Personalized therapy: The most obvious approach is to identify a biomarker (or biomarkers) allowing us to discriminate between the sensitive and unsensitive cancer cell lines. Whenever a tumor would score positive for that biomarker then we could use the corresponding treatment at a non-toxic dose. This strategy is off course more powerful when developed for a library of treatments, increasing the chances that most tumor types will score positive for the use of at least one treatment in the library. Using this type of approach we, in collaboration with others, have identified a small molecule with increased activity in cancer cells with a mutation in p53, one of the most common alterations in human tumors yet lacking fro a specific treatment (unpublished work).

Combination therapy: Another strategy is to use drug combinations, each at a non-toxic dose, such that together they target all or most cancer cell lines. We have recently shown that a three drugs combination is sufficient to target all the NCI60 cell lines, and there are 14 of such combinations [7], indicating that the drug combination approach is feasible and requires no more three-drug combinations. While the extrapolation of these observations to the cancer patient population is not straightforward, it provides a methodology to identify drug combinations that are efficient in the sense that each drug in the cocktail covers for the resistance to the others and are minimal by using the minimum number of drugs necessary to do the job.

Conclusions

This analysis demonstrate the power of looking at the response pattern of cancer and normal cell lines to a library of compounds, rather than just focusing on a few compounds manifesting the highest potency for selected cell lines. It reveals the major challenge in anticancer therapy: how to overcome the fact that cancer and normal cell line responses to an average treatment manifest overlapping distributions. Our results indicate that there is no single treatment targeting all cancer cell lines at a non-toxic dose. More important, we anticipate that both personalized medicine and combination therapy are feasible strategies for anticancer therapy at non-toxic doses. Finally, the indexes introduced here allow us to compare different treatments and rank them according to their therapeutic potential.

Methods

The datasets used in this study were obtained from: literature, author compilation; NCI60 screen, http://dtp.nci.nih.gov; Sanger screen, http://www.sanger.ac.uk/genetics/CGP/translation.

Acknowledgments and Funding

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