TIMELINE

The NCI60 human tumour cell line anticancer drug screen

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Abstract | The US National Cancer Institute (NCI) 60 human tumour cell line anticancer drug screen (NCI60) was developed in the late 1980s as an *in vitro* drug-discovery tool intended to supplant the use of transplantable animal tumours in anticancer drug screening. This screening model was rapidly recognized as a rich source of information about the mechanisms of growth inhibition and tumour-cell kill. Recently, its role has changed to that of a service screen supporting the cancer research community. Here I review the development, use and productivity of the screen, highlighting several outcomes that have contributed to advances in cancer chemotherapy.

The US National Cancer Institute (NCI) 60 anticancer drug screen was developed in the late 1980s with the aim of changing the emphasis of drug discovery from leukaemia, as modelled in transplantable murine neoplasms, to human solid tumours. This change was based on the relative lack of clinical activity of the compounds that had been identified using murine screens for the common human adult solid tumours, such as lung, colon, breast and prostate. The broad scope of the present-day NCI60 evolved from the narrower concept of an *in vitro* 'disease-oriented' screening model focused on lung cancer drug discovery. As lung tumour cell lines were acquired and assembled into a tumour panel, it rapidly became clear that some other cell types were necessary as controls. The finding that normal cell types available at the time, that is, fibroblasts and certain epithelial cell populations such as renal epithelial cells, responded *in vitro* to anticancer drugs with extreme phenotypes (fibroblasts being pan-resistant and renal epithelial cells being pan-sensitive) in the assay selected for the screen led to the use of other tumour cell lines as controls. As a result, panels of cell lines were assembled that ultimately represented nine distinct tumour types: leukaemia, colon, lung, CNS, renal, melanoma, ovarian, breast and prostate. Although the intention of the screen was to identify compounds with growth-inhibitory or toxic effects on particular tumour types (the disease-oriented concept), the patterns of relative drug sensitivity and resistance generated with standard anticancer drugs were rapidly found to reflect mechanisms of drug action. The information-rich character of the screening data therefore provided an additional and unexpected dimension to the screening model. Indeed, during more than a decade of use, the screen has produced a stream of discoveries that have contributed to fields such as targeted anticancer therapy using biological agents (immunotherapy), virology (HIV drug discovery and insight into viral pathogenesis), pathogenesis of bacterial toxins with bioterrorism potential, and molecular-targeted anticancer drug discovery. This article will describe the development and use of the screen and its productivity, highlighting examples of some of the important outcomes. The manner in which these discoveries evolved from the screening experience, and some of the informatic threads that tie them together, will be a focus of this Timeline article.

As indicated in the TIMELINE, the development and operation of the NCI60 has spanned more than 20 years. Work has proceeded through three phases: model development, operation as a primary drug screen and, most recently, operation as a screening service to the research community. Considerable discussion and planning preceded the initiation of work in the laboratory. A recently updated book chapter by Michael Boyd[1] describes this process, lists members of various *ad hoc* review committees convened to advise on the development of the NCI60 and acknowledges the important contributions of many, including Ken Harrap, who served as chairman on several occasions. Members of standing NCI advisory boards also provided significant input, notably Susan Horwitz and John Niederhuber. The inter-dependent nature of some of the main outcomes is indicated by colour coding on the TIMELINE. It is noteworthy that, for the most part, these events spanned several operational phases of the NCI60 and evolved over intervals of decades, attended by the generation of additional screening data, knowledge of molecular targets within the NCI60 and informatic tools for integrating information.

Assembly of the tumour cell line panels

By the mid 1980s many tumour cell lines had been established worldwide, and many were available from repositories such as the American Type Culture Collection. Indeed, an initial listing of ~1,500 cell lines was generated as potential source material for the proposed screen. However, it was also clear that there were potential problems inherent in using established tumour cell lines. The work of Walter Nelson-Rees and colleagues during the 1970s established that many tumour cell lines in relatively wide use as models of various tumours were, in fact, derived from the first immortal human cell line ever established — the HeLa cervical carcinoma cell line[2–4]. This observation was made possible by advances in cytogenetic techniques, notably chromosome banding, which enabled the characteristic HeLa marker chromosomes to be identified. Among others to be recognized as a HeLa derivative was the 'KB' cell line, which was used by the NCI for some of the earliest *in vitro* anticancer drug-screening work, and had been thought to be derived from an epidermoid carcinoma of the oral cavity[5]. In addition to concerns about the identify of cell lines, there was also concern about the authenticity of tissue origin, tumour

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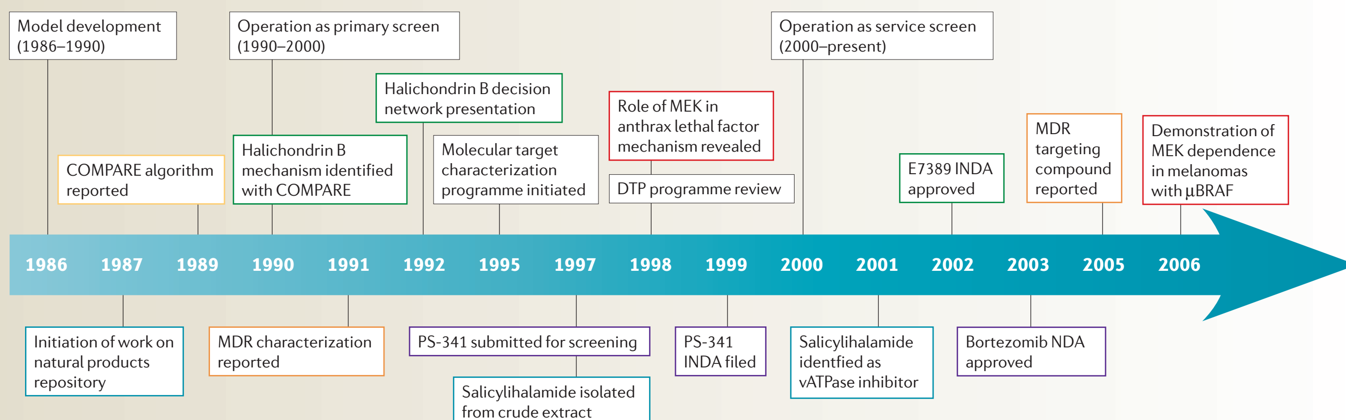
Timeline | **Major events in the development, implementation and use of the NCI60 cell lines**



Related events are indicated by colour coding. DTP, Developmental Therapeutics Program; INDA, investigational new drug application; MDR, multidrug resistance; MEK, mitogen-activated ERK kinase; NDA, new drug application.

type and the potential evolution of high-passage cell lines to a point at which they no longer represented the tumour as it existed in the patient. The general approach to the development of the tumour cell line panel has been previously reported[6].

A unique collection of well-characterized, low-passage lung cancer cell lines was established within the NCI Navy Medical Oncology Branch in Bethesda, beginning in the late 1970s[7–11]. John Minna, Adi Gazdar and colleagues generously shared this resource and their expertise in this disease area with staff of the Developmental Therapeutics Program at NCI Frederick to provide a firm scientific basis for the initiation of the 'Lung Cancer Drug Discovery Project' in autumn 1984. The many histological types and heterogeneity of lung cancer immediately posed challenges for the assembly of a representative cell line panel for use in drug screening. There were few well-differentiated squamous lung cancer cell lines available. By contrast, there were many non-small-cell lung cancer cell lines (adenocarcinoma, bronchioloalveolar and large-cell lung cancer), and many small-cell lung cancer lines, but these grew with fundamentally different geometry (multicellular aggregates) from the non-small-cell lung cancer lines and were not practical to use. Small-cell lung cancer 'variant' lines (DMS 114 and DMS 273) were obtained from Olive Pettengill and George Sorenson at the Dartmouth Medical Center, USA[12]. These grew as attached populations and were included in the initial version of the tumour cell line panel. The initial panel proved to be very useful for the development of assay methods, and was crucial in establishing the feasibility of the general approach.

As mentioned above, the use of normal cell populations as controls proved not to be feasible. Therefore, additional tumour cell lines were sought that might serve the purpose. The disease-oriented concept evolved to include panels of cell lines that were representative of eight additional tumour types. Again, the effort benefited from the generosity of many investigators with specialized expertise and cell line resources. Cell lines derived from CNS tumours were provided by Paul Kornblith, then at the Surgical Neurology Branch of the US National Institutes of Health (NIH), and Mark Rosenblum at the University of California at San Francisco. Unique cell lines were also provided by Isaiah Fidler, Oystein Fodstad, Heiner Fiebig, Marsten Linehan, Jean Benard, George Moore and Al Liebowitz. A detailed description of the cell lines included in the initial NCI60 panel, their sources and information regarding patient donors is given in Stinson *et al*[13].

Characterization of candidate cell lines

Cell lines that showed generally acceptable growth characteristics, including adaptability to the standard cell culture medium established for the project, were tested for sterility, including mycoplasma contamination, and then characterized cytogenetically by chromosome banding. This effort was specifically oriented toward identifying HeLa marker chromosomes. Although no instances of such markers were observed among the candidate cell lines acquired, the identity of certain cell lines was called into question. For example, the WiDr colon tumour cell line was shown, on the basis of G-banded chromosome analysis, to be derived from the HT-29 colon cancer cell line, and was therefore excluded from the screening panel. It is noteworthy that advanced genetic techniques such as restriction fragment length polymorphism analysis, DNA fingerprinting, spectral karyotyping and single nucleotide polymorphism analysis were not available in the late 1980s. These more sensitive techniques have subsequently been applied to the NCI60 and have shown additional instances of cell lines sharing a common origin, that is, SNB-19 is derived from U251 glioblastoma. In addition, the MDA-435 cell line and its ERBB2 transfectant MDA-N, originally thought to be from a breast cancer, seem to be melanoma in origin[14–15]. Both were apparently derived from the M-14 melanoma line[16]. Although the general drug sensitivity phenotypes of the M-14 and MDA-435 cell lines are similar, differences are evident and have presumably been caused by the further evolution of these genetically unstable populations. The identity of the MCF-7/ADR cell line as an MCF-7 breast cancer cell line derivative was first brought into question on the basis of DNA fingerprint evidence, and led to its re-designation as NCI/ADR[17]. Spectral karyotyping clearly shows that this cell line is derived from the ovarian cancer cell line OVCAR-8 (REF. 18). The robust multidrug resistant (MDR) phenotype of the *in vitro* drug-selected NCI/ADR cell line complements the naturally MDR cell lines such as HCT-15 colon and the renal cancer cell lines UO-31 and TK10, and was retained in the screening panel for that reason.

Because it was envisioned that members of the *in vitro* tumour cell line panel would be used as xenografts for experimental therapeutic studies, and to provide a means of morphologically characterizing their growth,

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candidates for inclusion in the screening panels were evaluated for tumorigenicity in athymic mice during the 1980s[19]. A broad range of growth characteristics was observed, with certain tumours showing very aggressive growth, for example, the NCI-H460 large-cell lung cancer, and others showing metastatic potential[20], as in the case of the LOX melanoma[21]. The quantification of metastatic tumour growth was later addressed with molecular[22] and serological[23] techniques in the 1990s and, most recently, with imaging approaches[24]. In recognition of the importance of the tumour microenvironment, considerable effort was devoted to establishing orthotopic transplantation models, particularly in the case of lung cancer[25].

Scope of the laboratory effort

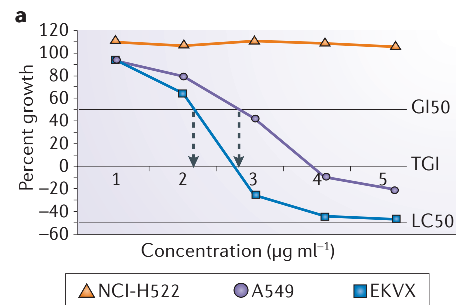
The design of the *in vitro* screening model required concentration–response testing for each compound in each member of the cell line panels, so as to assess the relative potency of test compounds across the cell lines. Chemically diverse compounds were acquired by the NCI for screening at the rate of 10,000 a year during the 1980s and 1990s. Using triplicate cultures for each concentration, five concentrations and 10,000 samples a year, the total number of culture units for the 60 cell lines was calculated to be 9,000,000 cultures a year. Testing on this scale was unprecedented, and clearly required miniaturization and automation to the maximum extent possible. Multi-well plastic tissue-culture dishes had come into routine laboratory use by the early 1980s, and 96-well plates were selected for use in the screen. For a detailed discussion of the logistic challenges involved in setting up the NCI60 see Boyd[1].

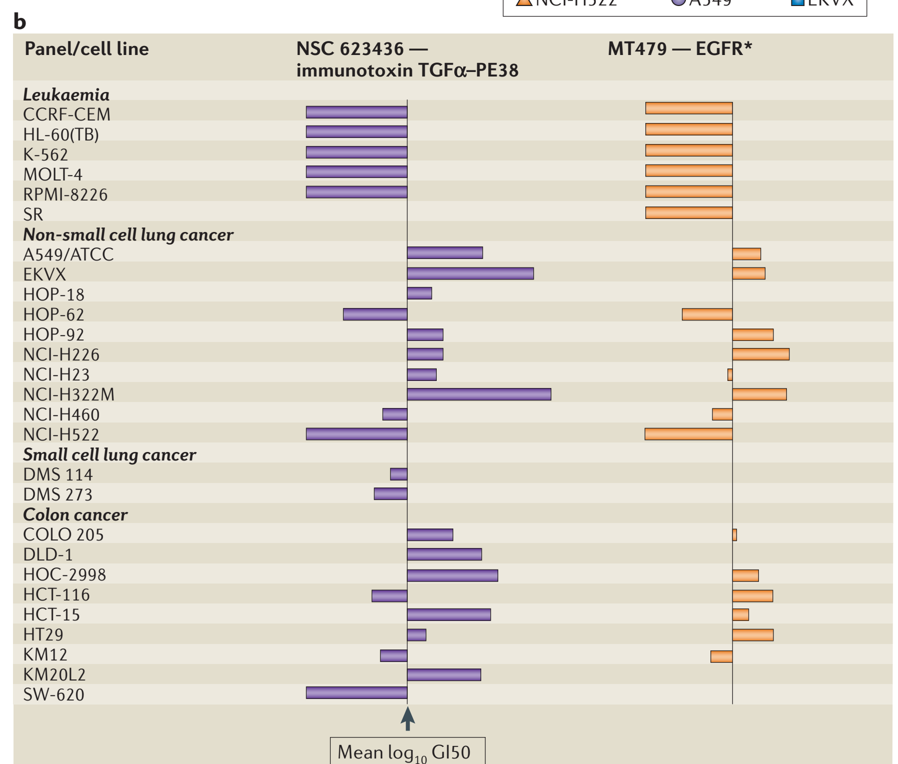
Assay development

Practical endpoints for microplate cytotoxicity assays did not exist at the time that the NCI60 model was conceived. The tritiated thymidine incorporation assay that was widely used at the time for growth-inhibition studies was prohibitively expensive for large-scale screening. A simple colorimetric (MTT) assay for use in immunology was introduced in 1983 (REF. 26), and seemed to be amenable for use in growth-inhibition assays. This technology, which relies on the metabolic reduction of a tetrazolium dye in viable cells to a coloured formazan product, was adapted and evaluated for growth-inhibition screening[27]. A limitation of the MTT assay is that the formazan product is insoluble in aqueous medium, so a step is required to solubilize the formazan before reading the optical density. To circumvent this, a new tetrazolium assay was developed

Box 1 | **Display and analysis of screening data from the NCI60**

When the US National Cancer Institute (NCI) 60 anticancer drug screen (NCI60) tumour cell lines are screened for the activity of test compounds, the resulting data can be displayed and analysed in various ways. For instance, the activity of one test compound on three non-small-cell lung cancer cell lines is shown in part **a** of the figure. The response parameters GI50 (50% growth inhibition) and LC50 (50% lethal concentration) are extracted from concentration–response curves by linear interpolation. TGI (total growth inhibition) is read as the x-axis intercept. Standard dilutions span a four-log concentration range, here: 1 = 1E-5.2, 2 = 1E-4.2, 3 = 1E-3.2, 4 = 1E-2.2 and 5 = 1E-1.2 (dilutions from a stock solution, expressed in μg ml^{-1}). Therefore, in this example for EKVX, the GI50 = 1E-4.05, the TGI = 1E-3.49 and the LC50 level of effect was not reached (>1E-1.2, the maximum concentration tested). Mean Graphs are constructed at each level of effect, with bars depicting the deviation of individual tumour cell lines from the overall mean value for all the cells tested. For purposes of illustration, only the GI50 Mean Graph for leukaemia, lung and colon cancer panels are shown in part **b** of the figure for two test compounds. Results (shown on the left) are for the immunotoxin transforming growth factor-α (TGFα)–PE38 (REF. 42) (NSC 623436), submitted by Ira Pastan in 1989. The profile of cell line response was consistent with expected expression of epidermal growth factor receptors (EGFR) to which TGFα binds in lung and colon carcinomas: leukaemic cell lines and small-cell lung cancers were not expected to express the receptor and were relatively unaffected by this chimeric toxin molecule. The Mean Graph based on relative mRNA levels for *EGFR*, shown on the right, mirrors the relative sensitivity of the panels to TGFα–PE38. Relatively sensitive lung cancer cell lines A549, EKVX and NCI-H322 show bars projecting to the right for both drug sensitivity and mRNA level for the EGFR target. The relatively resistant leukaemia cell lines all show mRNA levels below the NCI60 panel mean (bars projecting to the left). Automated COMPARE analysis provides a quantitative method for inter-relating cellular responses with the large database of molecular target information that has been amassed for the NCI60. The program produces rank-ordered lists of compounds or targets based on correlation analysis of the NCI60 profiles. In this example, molecular target information collected long after the original screening provided support for the original targeting strategy.





*The *EGFR* mRNA levels shown were generated using RNAse protection assays by Susan Bates, and are part of the publicly searchable database (designated MT479 in database).

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Figure 1 | **The halichondrin B team.** Photo (from left to right) of Robert Shoemaker, Ernest Hamel, George Pettit, Kenneth Paull and Michael Boyd at the NCI decision network meeting at which halichondrin B was presented as a drug-development candidate in March 1992.

based on a reagent designated XTT[28]. However, tetrazolium assays presented some practical challenges for large-scale screening[30]. Consequently, alternative approaches were investigated, including a methylene blue assay[31], and a method that used sulphorhodamine B (SRB) was finally selected for use in the screen. This assay[32] employed a chemical fixation step at the end of drug treatment that enabled the subsequent batch processing of plates in a time-independent manner. The assay also had the advantage of differentiating cell kill from growth inhibition. The SRB assay proved to be robust and feasible for large-scale screening[33–34]. The XTT assay served as an efficient bioassay tool for the isolation of natural products with anticancer potential, as described below, and provided the basis for the NCI anti-HIV drug screen[35–36].

Early findings

Profiles of cell line sensitivity provide information about mechanisms of growth inhibition and cell killing. The application of the SRB assay to the pilot-scale screening of test sets of compounds, including approved anticancer drugs, led to the recognition that the profiles of cell line sensitivity and resistance reflect mechanisms of growth inhibition and cell killing. Compounds that were known to function in a similar manner produced obviously similar patterns of activity. This observation prompted the development of formal conventions for the display and analysis of the screening data. The 'Mean Graph' provides a compact way of presenting the profile of relative sensitivity and resistance of all the cell lines at three levels of effect: 50% growth inhibition (GI50), total growth inhibition (TGI) and 50% lethal concentration (LC50), and the 'COMPARE' algorithm provides an automated way of comparing these profiles with all or parts of the screening database[37]. See BOX 1 for examples of data and explanations of methods. Mean Graph and COMPARE, which were initially developed and implemented on the Developmental Therapeutics Program (DTP) internal computer system under the energetic leadership of the late Kenneth Paull for use by NCI staff, were later incorporated into the DTP public website for use by the wider cancer research community. For a detailed description of the use and interpretation of COMPARE results, see Boyd and Paull[38]. The use of this program fueled interest in the development of additional bioinformatic approaches, both within and outside the NCI.

MDR phenotype leverages many profiles. An awareness of the importance of MDR in drug discovery and development led to the inclusion of the NCI/ADR cell line in the screening panels, as mentioned above. Instances of relatively dramatic resistance to classic P-glycoprotein (Pgp) substrates such as doxorubicin indicated that several of the other cell lines in the panel manifest a significant MDR phenotype. Early laboratory investigations of the NCI60 showed evidence of levels of Pgp expression that clearly correlated with drug resistance[39]. Functional studies of rhodamine and doxorubicin efflux provided further support for this relationship[40–41]. Several tumour cell lines, such as the HCT-15 colon and UO-31 renal lines, manifested distinctive MDR phenotypes associated with Pgp and MDRH associated protein[42] (MRP) . This early MDR characterization work set the stage for the identification of Pgp-targeting drugs over a decade later (see below). The clear association of cell response with MDR markers stimulated additional detailed studies of specific biochemical determinants of cellular response. In 1995, a formal programme for the molecular characterization of the NCI60 was established, together with the centralized DTP public web site for the deposition of characterization data generated by researchers throughout the community.

First disease-oriented drugs identified. In the early 1990s, the first class of compounds to show clear disease-oriented activity in the NCI60 were ellipticinium derivatives, which had selectivity for CNS-derived tumours[43]. Detailed studies of these compounds showed that selective drug uptake and processing had a role in CNS tumour selectivity[44]. Structure–activity studies were conducted and 9-Cl-2-methylellipticinium acetate was identified as a lead compound. This compound was shown to be capable of inhibiting the growth of subcutaneous and orthotopic glioblastoma xenografts[45], but the failure to cure most animals and the lack of activity in preclinical drug-combination studies[46] tempered enthusiasm for further development. For a review of other compounds identified by the NCI60 in the 1990s see Monks *et al*[47].

First application of the COMPARE algorithm to identify the mechanism of action of a natural product. Halichondrin B was submitted to the NCI60 by George Pettit of the Arizona

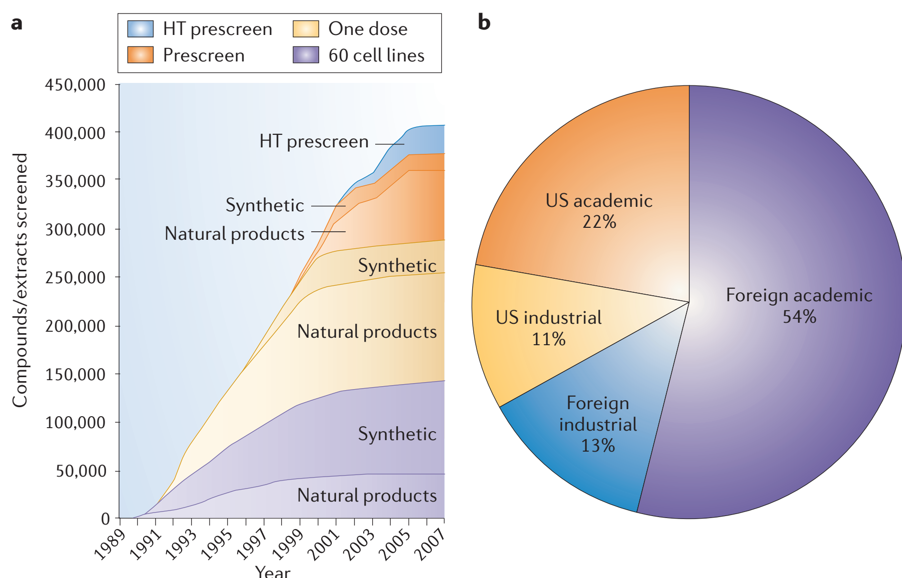

Figure 2 | **Sample throughput of the NCI60. a** | Illustration of the rate of testing and throughput of the US National Cancer Institute (NCI) 60 anticancer drug screen (NCI60) and associated prescreens used for synthetic compounds and crude natural product extracts. **b** | Analysis of the origin of submissions served by the screen in 1999–2005 for the past 40,000 compounds. HT, high throughput.

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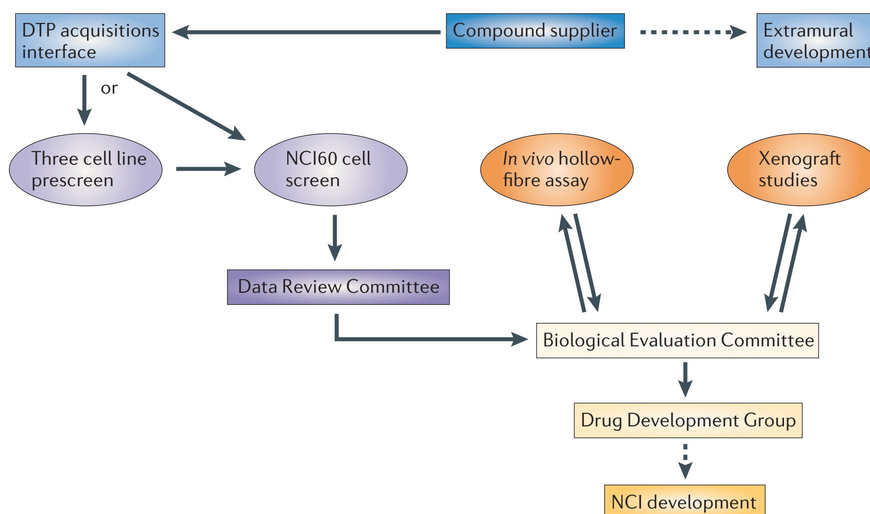
State University , USA, as an interesting natural product with an unknown mechanism of action. Using the COMPARE algorithm it was possible to associate the pattern of activity produced by this compound with known inhibitors of microtubule polymerization. Laboratory studies subsequently indicated that this compound does indeed interact with tubulin[48]. The OVCAR-3 cell line was particularly sensitive to this class of compounds, and was selected for *in vivo* experimental therapeutic studies. In this, and additional models, halichondrin B produced substantial anti-tumour activity[49]. On the basis of this activity, the compound was accepted by the Division of Cancer Treatment decision network committee for advanced preclinical development. FIG. 1 shows the 'halichondrin B team' at the NCI decision network meeting in March 1992. Although the compound was quite potent, it was clear that re-supply of this material from the natural environment could be challenging. Yoshito Kishi and colleagues at Eisai Research Laboratories, USA achieved a total synthesis of the molecule in 1998, and subsequently showed that a synthetic fragment of the molecule retained activity and was amenable to large-scale production[50–51]. Eisai 7389 produced a pattern of activity very similar to halichondrin B in the NCI60, and is currently under development.

Box 2 | NCI60 screening and secondary testing

Compound suppliers provide information about the biological rationale and chemical structures of compounds to be submitted for screening (see figure). Structures are examined and duplicates of previously screened compounds or representatives of well-studied chemical classes are rejected. The accepted samples are sent to a central repository and then to testing, either in a pre-screen model or directly to the US National Cancer Institute (NCI) 60 anticancer drug screen (NCI60). The results are then examined by a committee of scientists, and compounds that show a pattern of interest are retested. The Biological Evaluation Committee then considers the activity in the context of chemical and other available information. Compounds that have reproducible patterns of interest, usually those with a relatively unique, COMPARE-negative, profile of activity are initially tested in an *in vivo* hollow-fibre assay. Compounds that show significant activity in this model are then tested further in xenograft assays. Compounds with significant activity, and usually a lead as to their mechanism of action, can be presented to the Drug Development Group as candidates for NCI clinical development. Data returned to the compound supplier can be used for extramural development or licensing. DTP, Developmental Therapeutics Program.



Compounds from natural products

As the NCI60 was being developed, the DTP began amassing one of the world's largest collections of natural products[52,53]. The screening of crude extracts in the NCI60 began in 1989 (TIMELINE). In recognition of the fact that the screen did not have the capacity to test all of the available natural product extracts in a concentration–response fashion, and that most of the extracts did not cause sufficient growth inhibition to generate a useful pattern of activity, a single concentration prescreen using each cell line was designed and implemented in 1991 to identify extracts capable of producing a potentially useful profile in the full panel of cell lines. This streamlined procedure supported substantially greater throughput. A further streamlined prescreen that used only three cell lines was implemented in 1998. To date, more than 75,000 unique samples of plants, marine organisms and microbial samples have been collected, catalogued and extracted with aqueous and organic solvents to create a unique resource for drug discovery that is used by both NCI and extramural investigators. Throughput of both crude natural products and synthetic compounds in the NCI60 and associated prescreens is illustrated in FIG. 2a.

The Natural Products Branch (NPB) of the DTP has made extracts available to the research community. Many extramural investigators have isolated new bioactive molecules from this repository using various bioassays. Some recent examples include diazonamide A[54] (a tubulin binder), spirastrellolide A[55] (a protein phosphatase 2A inhibitor), a growth inhibitory 4-hydroxybenzoyl derivative[56], diterpenoid inhibitors of CDC25B phosphatase[57], psammaplysenes shown to be specific inhibitors of FOXO1A nuclear export[58], and cytotoxic brominated tryptophan alkaloids from two sponges of the family *Thorectidae*[59]. On the basis of unique patterns of activity in the NCI60, many bioassay-directed isolation efforts were pursued by NCI investigators, yielding more than 200 characterized compounds. A catalogue of these compounds can be found at the Molecular Targets Development Programme web site. New anti-tumour drug leads isolated by NCI investigators included the cytotoxics alertenone[60], halomon[61], lobatamides[62] and salicylahalamides[63]. Most of the NCI isolation work was performed using the XTT assay and a 2–10 cell line subset of the NCI60 tailored to the individual project, as illustrated in Bokesch *et al.*[60].

Although many new compounds were identified, perhaps the most interesting were the salicylahalamides. As a crude extract, the source material produced a unique profile in the NCI60. Bioassay-directed isolation yielded a new macrolide that was shown to function as an inhibitor of vacuolar ATPase[64]. Detailed biochemical studies have indicated that this effect engages a distinctly different mechanism from previously known vacuolar ATPase inhibitors such as bafilomycin[65].

Operation as a drug-discovery screen

As screening proceeded during the early 1990s, a substantial number of compounds were identified for *in vivo* testing. As a means of rapidly screening these, a new hollow-fibre implantation model was developed[66]. This involved loading tumour cell lines into biocompatible hollow fibres and implanting the fibres into mice, either subcutaneously or in the peritoneal cavity. Mice were

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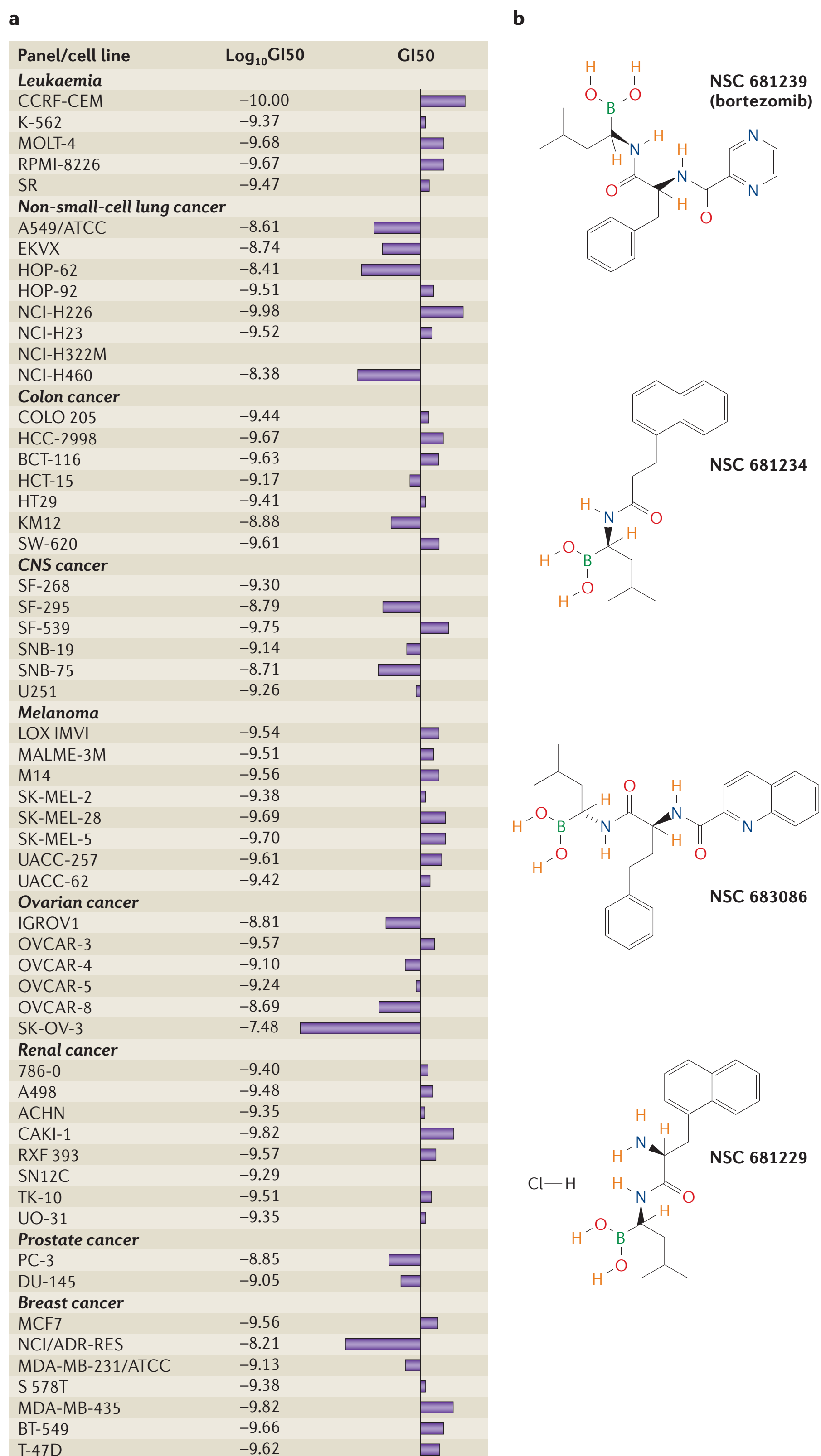

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Panel/cell line	Log_{10}GI50	GI50
Leukaemia		
CCRF-CEM	–10.00	
K-562	–9.37	
MOLT-4	–9.68	
RPMI-8226	–9.67	
SR	–9.47	
Non-small-cell lung cancer		
A549/ATCC	–8.61	
EKVX	–8.74	
HOP-62	–8.41	
HOP-92	–9.51	
NCI-H226	–9.98	
NCI-H23	–9.52	
NCI-H322M		
NCI-H460	–8.38	
Colon cancer		
COLO 205	–9.44	
HCC-2998	–9.67	
BCT-116	–9.63	
HCT-15	–9.17	
HT29	–9.41	
KM12	–8.88	
SW-620	–9.61	
CNS cancer		
SF-268	–9.30	
SF-295	–8.79	
SF-539	–9.75	
SNB-19	–9.14	
SNB-75	–8.71	
U251	–9.26	
Melanoma		
LOX IMVI	–9.54	
MALME-3M	–9.51	
M14	–9.56	
SK-MEL-2	–9.38	
SK-MEL-28	–9.69	
SK-MEL-5	–9.70	
UACC-257	–9.61	
UACC-62	–9.42	
Ovarian cancer		
IGROV1	–8.81	
OVCAR-3	–9.57	
OVCAR-4	–9.10	
OVCAR-5	–9.24	
OVCAR-8	–8.69	
SK-OV-3	–7.48	
Renal cancer		
786-0	–9.40	
A498	–9.48	
ACHN	–9.35	
CAKI-1	–9.82	
RXF 393	–9.57	
SN12C	–9.29	
TK-10	–9.51	
UO-31	–9.35	
Prostate cancer		
PC-3	–8.85	
DU-145	–9.05	
Breast cancer		
MCF7	–9.56	
NCI/ADR-RES	–8.21	
MDA-MB-231/ATCC	–9.13	
S 578T	–9.38	
MDA-MB-435	–9.82	
BT-549	–9.66	
T-47D	–9.62	

Figure 3 | **Illustration of NCI60 data for bortezomib (Velcade, PS-341, NSC 681239). a** | Mean Graph that illustrates the pattern of activity at the GI50 (50% growth inhibition) level of effect for bortezomib. **b** | COMPARE analysis of the synthetic compound screening database identified the three compounds on the right as having the most similar GI50 patterns to bortezomib (top). All three are structurally related boronic acids. For a detailed discussion of COMPARE results, see Holbeck and Sausville[81]. NCI60, US National Cancer Institute 60 anticancer drug screen.

then treated intraperitoneally with test compounds, and the effect on tumour cell growth was assessed on recovered cells by MTT assay. This assay was designed for completion in 1 week, decreasing the time required for conventional human tumour xenografts, which can require several months. Compounds that produced differential activity across tumour cell lines, or particularly potent, COMPARE-negative compounds were initially directed to the hollow-fibre model. Compounds that showed activity in this model were further evaluated in other xenograft models (BOX 2).

The role of the prescreen. As mentioned above, a three-cell prescreen for synthetic compounds was introduced in 1998. By instituting this prescreen, composed of the MCF-7 breast cancer, NCI-H460 large-cell lung cancer and SF-268 glioblastoma cell lines, which were selected to have maximal sensitivity on the basis of a retrospective analysis, testing in the NCI60 was focused on compounds that had the potential to generate useful profiles. Therefore, the efficiency and throughput of the NCI60 was considerably increased (FIG. 2a). In 2001, a high-throughput version of the prescreen was developed using 384-well plates and an Alamar blue (metabolic dye) assay endpoint. This homogeneous assay was highly amenable to automation, and so could support a very high testing rate. In addition to its use as an NCI60 prescreen, this assay was also used in high-throughput mode for a screening campaign of >80,000 compounds from the DTP Open Repository of synthetic compounds. This was conducted at a concentration of 2.5 µM, primarily to support users of the Open Repository who were engaged in molecular-targeted drug discovery by providing context in relation to the potential of these compounds to inhibit growth. Suppliers of the compounds tested in the screen have included both academic and industrial chemists in the United States and world wide. A breakdown of the source of the last 40,000 compounds acquired by the NCI is shown in FIG. 2b. The high proportion of foreign submissions was facilitated, in part, by the operation of an NCI European liason office in Brussels, and by the development of a web-based interface for compound submission.

Informatics beyond COMPARE

The successful application of the COMPARE algorithm to probing mechanisms of growth inhibition and cell killing, and the demonstration that certain molecular characteristics of tumour cell lines correlated with patterns of drug sensitivity and resistance, indicated

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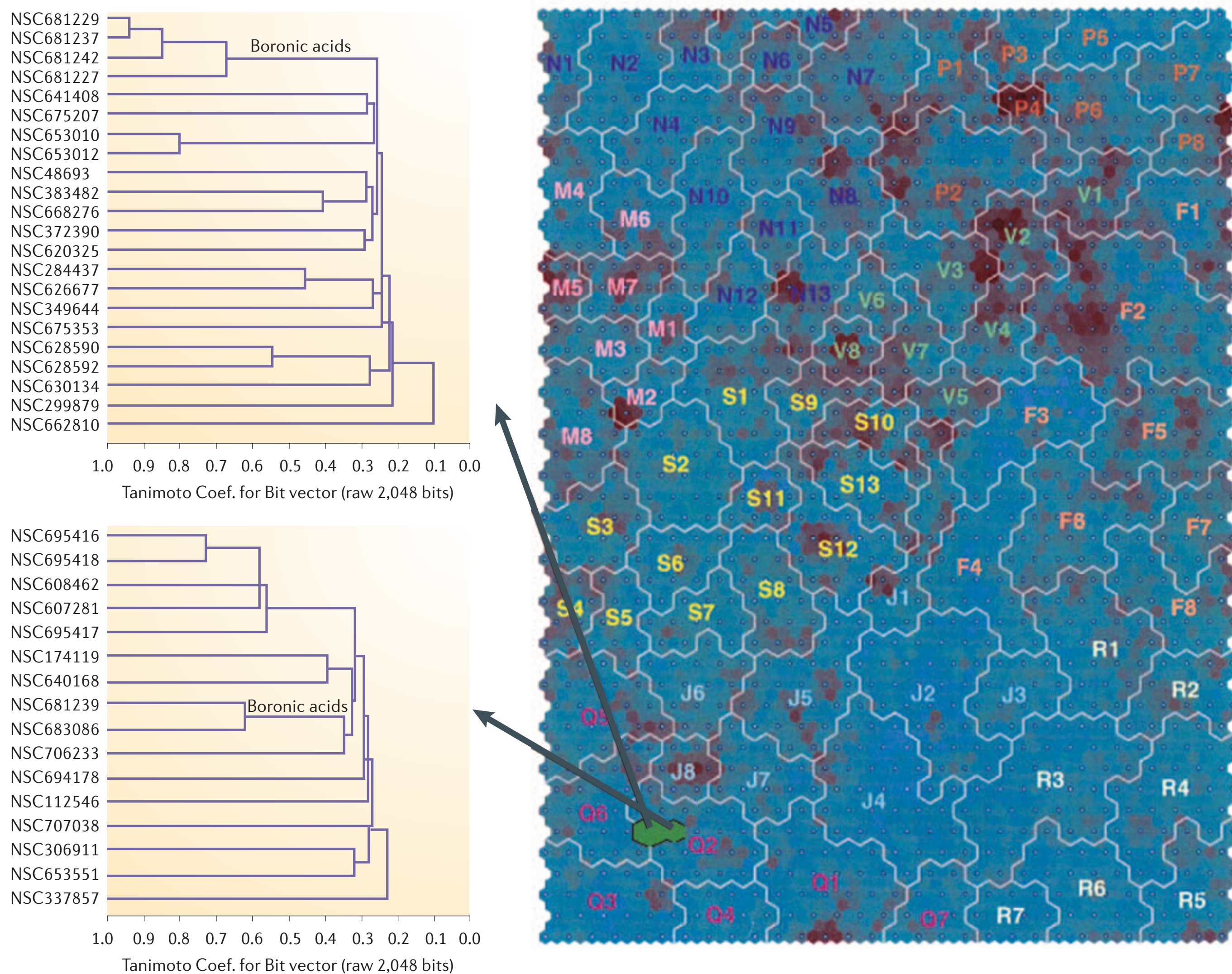

Figure 4 | **Self-organizing map (SOM) projections of bortezomib and structurally related boronic acids.** The SOM represents clusters of compounds that produce similar patterns of response across the US National Cancer Institute (NCI) 60 anticancer drug screen (NCI60). The SOM is available online at the 3D mind web site as an interactive tool for analysis and data mining. The dendrograms on the left (one of the online features) are built on the structural relatedness of the compounds within a node. Node 40.6 (green hexagons) contains bortezomib and NSC 683086, as well as other chemotypes that might have activity as proteasome inhibitors. The adjacent node in cellular response space contains four structurally related boronic acids and various other chemotypes, which might also act as proteasome inhibitors.

that the integration of additional information about molecular characteristics could yield even more insight into opportunities for cancer treatment. John Weinstein's group at the NCI pioneered the first such efforts in the early 1990s and, among other accomplishments, linked gene-expression information to *in vitro* screening data[14,15,67–69]. This prompted the group to develop clustered heat maps[69], which have since become one of the most frequently used visualizations for genomic, proteomic and high-throughput pharmacological data. The data-mining website created by the Genomics and Bioinformatics Group also provides tools for interpreting and integrating genetic information. As an example of the power of this approach, the profiles of basal gene expression provided the first indication that the MDA-435 breast cancer cell line might, in fact, be a melanoma. In the extramural cancer research community, Xueliang Fang and Shaomeng Wang have created an interactive Databases Correlation web site at the University of Michigan that supports COMPARE and related analyses. David Covell and colleagues in the Screening Technologies Branch of the DTP have created a set of publicly accessible data-mining tools built on neural networks and self-organizing maps (SOM), which complement COMPARE and other analytical approaches[70–73].

Response profiles provide insight into the mechanism of cytotoxicity of anthrax lethal factor. George Vande Woude's lab at NCI Frederick submitted recombinant anthrax lethal factor to the NCI60 screen in May 1995. The pattern of cellular sensitivity was found using COMPARE to correlate closely with that produced by PD98059, a known inhibitor of the mitogen-activated ERK kinase (MEK) pathway[74]. This result provided the first insight into the mechanism of cell killing by this toxin[75–77], and helped define a molecular target for high-throughput anti-toxin screening[78]. The anthrax lethal factor and PD98059 patterns in the database supported a detailed analysis of cell signalling in melanomas

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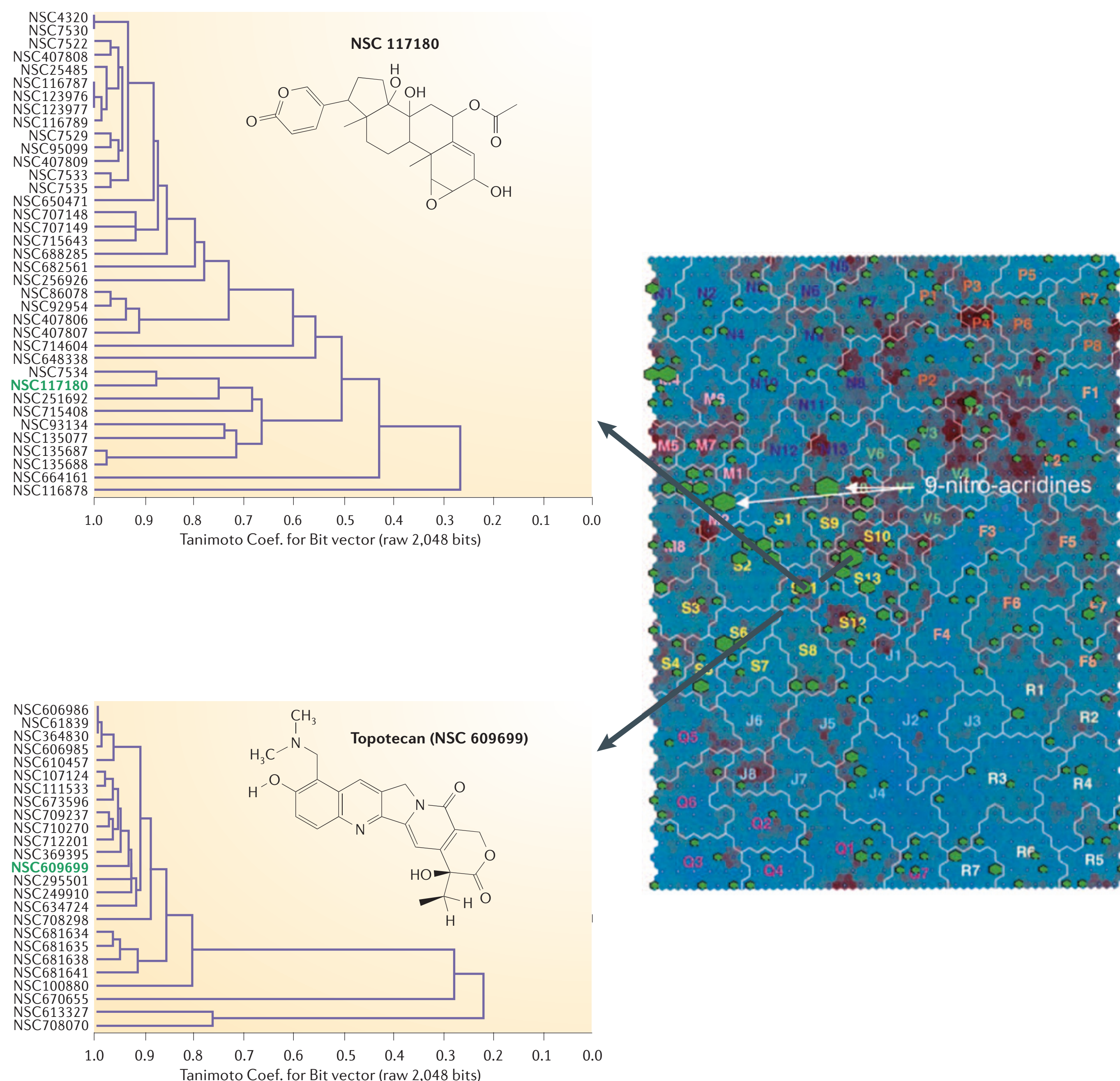

Figure 5 | **Self-organizing map (SOM) projections of hypoxia inducible factor 1α (HIF1α) high-throughput screen active compounds.** The 1% most inhibitory compounds observed in a screen of 151,320 compounds in the Developmental Therapeutics Program (DTP) Open Repository were projected onto US National Cancer Institute (NCI) 60 anticancer drug screen (NCI60) response space using the public web tools at the 3D mind website. Of these 1,513 compounds, data from previous testing in the NCI60 was available for 264. The location of these compounds in response space is indicated by light green hexagons, with the size of the hexagon reflecting the number of compounds in the node. The occurrence of clustering provides potential insight into the mechanistic basis for activity in the cell-based HIF1α high-throughput screen. Significant clustering is observed in the four clusters annotated on the figure. Two nodes containing 9-nitro-acridines are indicated. These were found to be inhibitory to the HIF1α high-throughput screen owing to cellular toxicity. Node 22.13 contained many camptothecin derivatives, including topotecan (NSC 609699, structure shown). The dendrogram illustrates the chemical relatedness of the compounds included in this node. Camptothecin-type DNA topoisomerase I (TOP1) inhibitors were the first active class of compounds identified with this high-throughput assay in a pilot-scale screen of the DTP Diversity Set[84]. Detailed studies have established the key role of TOP1 in the mechanism of HIF1α inhibition by such compounds[85]. Topotecan exerts its effect on HIF1α at relatively non-toxic concentrations *in vitro* and *in vivo*[85]. Giovanni Melillo has recently initiated a clinical trial of topotecan as an HIF1α inhibitor at the US National Institutes of Health Clinical Center. Node 24.10 contained bufadieneolides (the structure of NSC 117180 is illustrated) and closely related compounds. Some, such as NSC 117180, produced HIF1α inhibition at relatively non-toxic concentrations, and were therefore of potential interest as drug leads. Chemoinformatic investigation of members of this node rapidly identified nerifolin (NSC 123976) as a very potent inhibitor of HIF1α signalling. Neriifolin had been isolated as a purified natural product based on the NCI60 activity of a crude plant extract[86], and was considered for development by the NCI in the late 1990s. Unfortunately, cardiotoxic effects observed in preclinical toxicology studies precluded further development. Mechanistic clues for smaller clusters and individual compounds can be similarly inferred by their location in particular regions of the SOM[71].

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reported nearly a decade later with special reference to tumours with mutant *BRAF* genes (see below).

Support for the development of bortezomib. Perhaps the most notable contribution of the NCI60 to current cancer chemotherapy was the development of bortezomib (Velcade; PS-341), which was approved by the US Food and Drug Administration (FDA) in March 2003 for use in the treatment of myeloma. This compound was submitted to the screen, together with a group of chemical analogues, by Proscript Pharmaceuticals in July 1995. The pattern generated in the screen was COMPARE-negative, which indicated a mechanism of action distinct from known anti-tumour drugs. Follow-up testing in the hollow-fibre model showed activity for the chemical class that was confirmed in xenograft studies. Emerging information about the mechanism of proteasome inhibition[79] fuelled interest in clinical testing[80]. It took 8 years from initial testing in the NCI60 to FDA approval, probably making bortezomib the most rapidly developed new anticancer drug in recent history. The GI50 profile of bortezomib in the NCI60 is illustrated in FIG. 3. In this figure also shows related boronic acid compounds identified by COMPARE and the complementary analysis provided by interactive SOMs is shown in FIG. 4. For additional information on the application of COMPARE to bortezomib, see Holbeck and Sausville[81].

Transition of the NCI60 to a research tool

Beginning in 1997, a year-long, comprehensive external review of the DTP was conducted. The review (titled the Report of the NCI DTP Review Group) led to a number of recommendations for changes to the DTP. The review resulted in a change in the mode of operation of the NCI60 from an NCI drug-discovery pipeline to a research tool in support of the cancer research community. It was also recommended that the DTP develop capabilities for high-throughput, molecular-targeted screening, and increase connections with the extramural research community. Therefore, in 2000, resources were diverted from the NCI60 to support molecular-targeted screening and the Rapid Access to NCI Discovery Resources (R*A*N*D) programme was initiated to provide a peer-reviewed means of connecting DTP discovery resources with the most meritorious new molecular targets and discovery projects envisioned by extramural researchers. A detailed description of the R*A*N*D programme, including specifics about supported projects, can be found on the dedicated R*A*N*D section of the DTP website. Resources for NCI60 operations were significantly reduced at this time, and further reductions have resulted in a current workforce of about 20% of that which was employed when the screen was fully implemented in 1990. Screening throughput was reduced to around 3,000 samples a year, as indicted by the inflection in the cumulative testing curve illustrated in FIG. 2a.

Identification of P-glycoprotein-targeting compounds. The comprehensive molecular characterization of ATP-binding cassette transporters across the NCI60 panel, reported in 2004 as a collaborative effort between Michael Gottesman's and John Weinstein's laboratories, has supported the identification of the transporters that are most important for *in vitro* drug resistance[82]. Supplementary information to their research paper is publicly available on the *Cancer Cell* website. Data mining using this information identified a thiosemicarbazone (NSC 73306), which was screened in 1996, as a drug lead for targeting MDR tumour cell populations. Detailed studies have shown that this compound is selectively toxic to Pgp-expressing tumour cell populations *in vitro* and in xenograft models. This compound is currently undergoing advanced preclinical testing by the NCI.

This project can serve as a template for investigators who are interested in other molecular targets. If the target has already been characterized in the NCI60, the screening database can be mined for compounds that could affect the target using COMPARE tools on the DTP website. Transcriptional data are currently available for many thousands of genes, and can be the starting point for data mining. In hundreds of cases, protein levels or functional information are also available. If information is not available, investigators can access cell or nucleic acid samples from the NCI60 through the DTP molecular targets programme and generate the necessary data themselves. Samples of compounds found to COMPARE with the target profile can be requested from the DTP Open Repository and tested in the investigator's laboratory to verify molecular targeting.

Identifying inhibitors of mutant* BRAF *signalling from the public database. In 2005, Neal Rosen, Levi Garraway, William Sellers and collaborators conducted database mining focused on the molecular targets database to identify drug leads to target tumour cells that expressed mutant BRAF, a prominent target in malignant melanoma[83]. Remarkably, this project builds directly on the anthrax lethal factor story described above, in that the PD98059 MEK-inhibitor profile used to help decode the lethal factor mechanism also proved to be a potent inhibitor of growth of tumour cell lines that expressed mutant BRAF. Hypothemycin and CI-1040 were also found to inhibit such cells, which have a dependency on MEK for growth. This *in vitro* selectivity was shown to translate into *in vivo* xenograft efficacy, which supported the further development of MEK inhibitors for melanoma treatment. This identification of 'disease-oriented' activity in melanoma provides support for the validity of the early concept of the NCI60, and clearly links it to molecular determinants of drug sensitivity within the members of the tumour cell line panels.

Adding to molecular-targeted screens

The NCI60 can complement molecular-targeted, high-throughput screens in various ways. The testing of compounds with known targeting characteristics ('seeds' for the COMPARE algorithm) can identify the mechanistic basis for otherwise enigmatic clusters in cellular-response space. Knowledge of the mechanistic association of the cluster can enable the identification of analogues or other chemotypes that share mechanistic activity that could have advantages for development. FIG. 3 shows this in the case of proteasome inhibitors.

In cell-based, high-throughput molecular-targeted screens, compounds can potentially function in various ways to activate reporters. Molecular action in any of several 'upstream' pathways could result in activity. Identifying the detailed mechanism of action can be a very challenging laboratory task. In instances where such active compounds have been tested in the NCI60, informatic analysis can give leads about such mechanisms. The use of the SOM tools described above can facilitate the dissection of molecular mechanisms through the association of subsets of active compounds into mechanism-related clusters, known as response spaces. For example, inhibitors of hypoxic cell signalling identified in a cell-based screen[84] are projected on NCI60 response space in FIG. 5. This procedure yields clustering and single compounds distributed across response space. The most prominent clustering is associated with DNA topoisomerase I inhibitors such as camptothecin. Indeed, camptothecin-type compounds were the first to emerge from this screen in 2004 as selective inhibitors of hypoxia inducible factor 1α (HIF1α)[84,85]. The other notable cluster was identified as containing bufadienolides. Many structural

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analogues of the HIF1α-active bufadienolides identified in the high-throughput screen are therefore implicated as mechanistic analogues. The cardiac side effects of these compounds, as illustrated by neriifolin, a prototype bufadienolide isolated as a natural product from the NCI Open Repository and considered for development by the NCI in the 1990s[86], could be used to de-prioritize these as drug-development leads for HIF1α targeting. Although the example chosen here derives directly from NCI screening experience, the public availability of the data-mining tools used here shows their general value to the cancer research community. Molecular-targeted screening using chemical libraries distinct from the NCI Open Repository can still benefit from these tools, as chemical analogues of leads or compounds of interest can frequently be found in the NCI60 screening database.

Reduced cell panels support COMPARE

Takao Yamori and colleagues at the Cancer Chemotherapy Center in Tokyo began establishing a 39 human tumour cell line screen in the early 1990s using the methods and some of the tumour cell lines used in the NCI60, supplemented with gastric carcinoma and other cell lines of particular interest in Japan. Using this model, they have been able to implement the COMPARE analysis in a way that supports mechanism of action determinations as well as more advanced data mining[87–90]. As a recent example, published in 2006, these investigators identified a new chemical class of inhibitors of phosphatidylinositol-3-kinase (PI3K) based on the similarity of the profiles to the prototypical inhibitors wortmanin and LY294002. The new inhibitor identified in their screen, ZSTK474, has shown oral bioavailability and anti-tumour efficacy in mouse xenograft models[91].

Future prospects

The continuing flow of discoveries enabled by the NCI60 argues in favour of continued support for this unique resource. Although the throughput for the screen has been curtailed, concomitant with re-allocation of NCI resources to other areas, the available public database remains the largest source of cell-based anticancer testing data. The NCI60 database was one of the first to be incorporated into the overarching informatic initiatives of the US National Library of Medicine. Chemistry and biological activity in the NCI60, and other NCI efficacy models, is accessible through PubChem.

Fully exploiting the NCI60 requires a capability for the prospective generation of new profiles for compounds of interest. With resources currently reduced to about 20% of the level applied to the effort during the 1990s, continuing operations is a challenge that requires increasing efficiency and attention to detail.

Increased automation, micro-sizing to 384-well plates and reducing the number of cell lines in the NCI60 are approaches to streamlining the screen. New commercial automation equipment, acquired by the DTP for molecular-targeted, high-throughput screening, can perform some of the labour-intensive steps of the screen such as the addition of test samples. Preliminary results even indicate superior reproducibility when this is done by machine. Reducing the size of cultures and increasing density from 96- to 384-well plates could reduce cost and increase efficiency. The use of 384-well plates would have the added advantage of supporting additional quality control by providing inbuilt positive control wells. As described above, Yamori *et al.*[87–91] have set the precedent for the use of reduced numbers of cell lines at the same time as maintaining the information-rich character of the screen. Simulations performed on the NCI screening database also indicate that smaller numbers of cell lines can be used. However, any reduction in cell number is associated with some decrease in pattern resolution. Although these various options offer ways of increasing the efficiency of screening operations, it is clear that this would not be of value unless the data generated could be used in conjunction with the database amassed by previous screening. Future developments must keep this objective as a top priority.

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Competing interests statement
The author declares no competing financial interests.

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