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The hollow fibre model in cancer drug screening: the NCI experience

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Abstract

The *in vivo* hollow fibre model was developed by the National Cancer Institute (NCI) in the United States of America (USA) at a time when the number of potential anti-cancer drugs arising from *in vitro* screening efforts exceeded the available capacity for testing in traditional xenograft models. Updated analysis of the predictive value of the hollow fibre model continues to indicate that the greater the response in the hollow fibre assay, the more likely it is that activity will be seen in subsequent xenograft models. The original 12 cell line hollow fibre panel has been supplemented with histology-specific panels, and we begin here to analyse their utility in predicting activity in subsequent *in vivo* models. The key goal of using the hollow fibre model as a way to decrease the cost, both financial and in the number of animals used, to evaluate initial evidence of a compound's capacity to act across physiological barriers continues to be reinforced with our enlarging experience.

Keywords: Hollow fibre; Drug discovery; Drug screening; *In vivo* models

1. Why use hollow fibres?

New anti-cancer drugs arise from two predominant sources. *Empirical* screening against proliferating cell models either *in vitro* or, more unusually at this time, *in vivo* models provide lead structures whose further capacity to act *in vivo* to any degree, or against a potential range of cell types, is of interest to approach before considering further developmental efforts. *Targeted* screening strategies seek to elaborate lead structures against defined molecular targets acting either in fully *in vitro* biochemical screens or in cell-based assays, and also *in vitro* in tissue culture systems. Again, the capacity of lead structures to act in the *in vivo* milieu could be of value in selecting from among lead structures for further optimisation.

Commencing in 1990, the National Cancer Institute (NCI) in the United States of America (USA) undertook the operation of a 60 cell line *in vitro* screen to define novel anti-proliferative agents with either selectivity for different disease histologies, differential activity manifest against cell types of diverse histologies, or potent capacity to affect solid tumour-derived as well as haematological neoplasm-derived tumour cells. The details of the operation of this screening system, and the value of bioinformatic approaches to mining the data produced, have been described previously in Refs. [1,2]. Soon after that screening process began to generate candidate anti-proliferative leads, further evaluation in *in vivo* models of the compounds identified as possessing some evidence of anti-proliferative activity *in vitro* was identified as the next step prior to further development. However, the cost, time, and expense of running conventional xenograft models with empirical dosing strategies for all such lead compounds, or developing pharmacokinetic assays for each compound to be evaluated *in vivo,* would in either case greatly reduce the rate at which compounds could be evaluated.

To address this problem, a short-term *in vivo* assay was devised [3] in which cells growing in polyvinylidene fluoride (PVDF) "hollow fibres" are placed in various body compartments of mice. These fibres are permeable to substances with a molecular weight $<500\,000$. Compounds can then be dosed at intervals over a 4–5 day period, as practiced at the NCI, and then the fibres removed from the mice and the effect of compound action assessed by colorimetric assays with a tetrazolium-based dye. This assay was validated with a "training set" of standard agents and a scoring system

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defined, which is described in detail below, that is biased to result in detecting as "positive" all of a panel of standard anti-cancer agents.

An initial evaluation of the ability of the hollow fibre assay to successfully define compounds which go on to show evidence of *in vivo* activity in conventional athymic mice bearing xenografts of human tumours has been published in Ref. [4] and will be updated here. In general, using the scoring algorithm described in detail below for 12 cell lines placed in both intraperitoneal (i.p.) and subcutaneous (s.c.) compartments, the greater the number of fibres demonstrating evidence of an anti-proliferative effect, the greater the likelihood that a compound will display activity against xenografts.

The hollow fibre assay is not intended to replace detailed biological models such as transgenic or knockout models that allow insights into biology and pathogenesis. It also uniquely does not score aspects of the host stromal or immunological responses, although others have shown that prolonged residence of fibres in certain murine strains can evoke a noteworthy pro-angiogenic response [5]. The principle value of the hollow fibre model is its serving as an initial point of triage to indicate which compounds should be studied further in detailed *in vivo* model studies. It can be positioned to evaluate a stream of screening leads and maintain a higher level of throughput than if the initial *in vivo* evaluation consists of syngeneic or xenografted cell types. In addition, the ability of the hollow fibres to provide cells for various types of biological analyses after retrieval of cell-bearing fibres from the drug-treated host allows the possibility of scoring molecular endpoints of drug action at early times after drug treatment [6]. In principle, this would also allow correlation with intrafibre concentrations of administered drug assuming an assay of sufficient sensitivity were available. At the very least, when coupled to a consideration of the anti-proliferative effects of administered doses, hollow fibre assay data can provide a rapid initial assessment of the ability of a lead structure to distribute from compartments of administration across various types of physiological and membrane barriers.

2. Process and scoring of the hollow fibre assay

In the hollow fibre assay, tumour cells are inoculated into hollow (1 mm internal diameter) PVDF fibres, and these are heat-sealed and cut at 2 cm intervals. The fibres are cultured for 24–48 h *in vitro* and then implanted into athymic (nu/nu) mice. Although it is true that non-immunocompromised mice could (in principle) be used in this assay, since further *in vivo* evaluations in the event of a "positive" hollow fibre assay utilise athymic mouse xenografts, use of the same strain facilitates pharmacology and metabolism issues allowing easier interpretation of the hollow fibre results. Each mouse receives a total of six implants, consisting of three fibres placed in the peritoneum (i.p.) and three fibres placed in the s.c. compartment. There are three mice per treatment group and six mice per vehicle control group.

At the time of implantation, a representative set of fibres is assayed for the viable cell mass by the "stable-endpoint" dimethylthiazolyl-2,5-diphenyltetrazolium bromide (MTT) dye conversion technique [7] to determine the time zero cell mass for each cell line. The mice are treated with test agents on a daily treatment schedule (QD×4). Agents are tested at two dose levels and are typically administered i.p. The fibres are collected 6–8 days post-implantation. At collection, the quantity of viable cells contained in the fibres is measured by the "stable-endpoint" MTT dye conversion assay. The antitumour effects of the test agent are determined from the changes in the viable cell mass in the fibres collected from compound-treated and diluent-treated mice. The agent is considered to have an effect on a particular fibre if there is a 50% or greater reduction in net cell growth compared with the controls [3].

Sets of hollow fibre screening panels were established to accommodate the testing of agents with different expected activities (Table 1). The standard assay uses 12 human tumour cell lines, representing six different histologies, selected owing to the expected behaviour of their corresponding xenografts. In addition, panels representing specific histologies are used for specialised

Table 1
Hollow fibre screening panels

Standard		Leukaemia/ lymphoma	Melanoma	Prostate	Renal
Breast	*Colon*	HL-60	MALME-3M	JCA-1	RXF-393
MDA-MB-231	COLO-205	MOLT-4	M14	DU-145	A498
MDA-MB-435	SW-620				
Glioma	*Melanoma*	AS-283	SK-MEL-2	PC-3	CaKi-1
U251	LOX-IMVI	KD488	SK-MEL-5		
SF-295	UACC-62				
Ovarian	*Lung*	SR	SK-MEL-28		
OVCAR-3	NCI-H23	RPMI-8226	UACC-257		
OVCAR-5	NCI-H522				

testing of compounds on a non-routine basis. To simplify the evaluation of the hollow fibre assay, a scoring system was developed in which a compound is given 2 points for each fibre in which there is a 50% or greater reduction in net cell growth compared with the controls (Table 2). In this scoring system, the maximum total score a compound can achieve in the standard hollow fibre assay is 96 (12 cell lines×2 implant sites×2 dose levels×2 points/positive fibre). However, the score is typically recorded as the score in the i.p. fibres + the score in the s.c. fibres (e.g. 48 + 48). Although not accounted for in the score, the capacity of the compound to produce cell kill, defined as a lower viable cell mass at the end of the incubation compared with time 0, is also recorded.

When the standard hollow fibre assay was established, the criteria for activity were based, and statistically validated, on the scores achieved by clinically-used anticancer agents. Compounds meeting any of the following criteria were considered for follow-up xenograft testing:

total (i.p. + s.c.) score of 20 or greater;
a s.c. score of 8 or greater;
cell kill in any cell line at either dose level evaluated.

3. How the NCI uses hollow fibre data in decision-making

Initial evidence of anti-tumour efficacy according to the above criteria can be used in several ways, and there has been a continuing evolution over the past decade in the subsequent steps. Initially (1995–1999), positive hollow fibre results were the trigger for a series of xenograft studies in a range of histologies encompassing the responding cell types in hollow fibres. However, as a result of continuing review and advice from extramural advisory panels, development of anti-tumour agents purely on the basis of empirical anti-tumour activity in human tumour xenografts without definition of either a compound's molecular target or a set of biological correlates of drug efficacy has been abandoned [8]. Accordingly, a major use of positive hollow fibre results has been to prioritise compounds for further pharmacological and mechanistic studies. Specifically, activity in hollow fibres is accompanied by an effort to define the pharmacology (peak concentrations and area under the concentration×time curve) associated with antiproliferative action. This is modelled *in vitro,* and these results used to either correlate with a putative target of drug action, or to devise a pharmacodynamic correlate of drug effect that in principle could be extended to further development steps including use in evaluating the results of early clinical trials. An additional use of the hollow fibre information is to select among a series of congeners to evaluate the potential for best *in vivo* activity, or to modulate the appearance of cytotoxicity of concomitantly administered "standard" agents in the case of drug resistance modulators. The above activities represent Stage IB of the NCI's decision-making algorithm that is presented in detail on http://dtp.nci.nih.gov/docs/ddg/ddg_descript.html. These data are then considered with initial pharmacokinetic information obtained from animals dosed by various routes with the new compound to decide how best to approach formal xenograft studies. These are ideally undertaken only with models that express the molecular target of the drug's effect, and utilise schedules of drug administration known to be able to affect the intended target or cellular endpoint.

Compounds for which a cogent rationale for further development is elucidated are advanced to subsequent toxicology evaluations. This generally includes use of drug schedules imparting activity in xenograft models in a way that is tied to well-understood pharmacology in the test species and to documentation of a molecular or biological effect that can be tied to the anti-proliferative mechanism of the compound under study. The hollow fibre model therefore serves as a key "gate-keeper" in decision-making for subsequent more detailed *in vivo* evaluations.

4. Update on the hollow fibre predictive value

Previously, we had described results indicating that agents that had activity in more than one-third of tested xenograft models were more likely to demonstrate

Table 2
Scoring system for hollow fibre assay

	Standard assay	Leukaemia or melanoma assays	Prostate or renal assays
(a) # of cell lines	12	6	3
(b) # of implant sites (i.p., s.c.)	2	2	2
(c) # of dose levels	2	2	2
(d) # of points per fibre	2	2	2
Maximum possible score (a×b×c×d)	96 (48 i.p. +48 s.c.)	48 (24 i.p. +24 s.c.)	24 (12 i.p. +12 s.c.)

#, number; i.p., intraperitoneally; s.c., subcutaneously.

activity in at least one Phase II trial than those agents that did not attain that level of xenograft activity [4]. Since, as we describe above, hollow fibre data are used by NCI in the determination of whether an agent merits testing in these xenograft models, it is desirable to have a detailed understanding of the hollow fibre assay's ability to predict activity, defined here as a T/C (treated tumour weight/vehicle control tumour weight) of $\leqslant 40\%$, in multiple xenograft models. To provide here an update of our previous work, we considered the i.p. hollow fibre score and xenograft activity of 690 compounds tested in both types of assays. Table 3 indicates that the greater the i.p. hollow fibre score, the greater the likelihood that an agent will be active in at least one-third of the xenograft models in which it is tested, 27% for agents with an i.p. score of at least 14 versus 9.5% for those with an i.p. score between 0 and 6 ($P < 0.0001$, χ^2 test).

Most of the compounds analysed above, 438 out of 690, had no activity in any of the xenograft models tested. To rule out the possibility that these were skewing the analysis, we removed these 438 compounds and considered whether the hollow fibre assay was still predictive of whether agents were likely to be active in multiple xenograft assays. As shown in Table 4, 53% of agents that had an i.p. hollow fibre score of at least 14 also had activity in more than one-third of the tested xenograft models, whereas only 33% of these with an i.p. score of 0–6 did ($P = 0.011$, χ^2 test).

Tables 3 and 4 are useful for predicting whether an agent is likely to attain a level of xenograft activity concordant with more likely clinical activity. For earlier decision-making purposes, we also consider it useful to know whether the hollow fibre assay is predictive of whether an agent is likely to have activity in any xenograft model at all. In Table 5, one again sees that the greater the i.p. hollow fibre score, the greater the likelihood of activity in at least one xenograft model.

The above analyses are based on comparisons of activity of i.p. delivered drug to i.p. hollow fibres with activity in any type of xenograft model. However, a more specific question, and one that allows for less influence from pharmaceutical issues, is whether activity in i.p. implanted hollow fibres is predictive of activity in i.p. implanted xenografts. Table 6 indicates that, in fact, the larger the number of i.p. hollow fibres with activity, the greater the likelihood of activity in an i.p. xenograft ($P < 0.0001$, χ^2 test).

Having asked the specific question of whether drug delivered i.p. to an i.p. hollow fibre is predictive of activity in a xenograft model, we returned to the original design of the hollow fibre assay with fibres also implanted in the s.c. compartment. The intent in this design was to use activity in the s.c. fibres from an i.p. delivered drug to serve as at least a crude indication of a test agent's ability to travel from the injection site to a distant tumour location. However, in previous work, we

Table 3
Intraperitoneal (i.p.) hollow fibre activity versus xenograft activity in at least one-third of tested models

IP score	% Of xenograft models active		Total	% Active in 33% of xenografts
	<33%	⩾33%		
0–6	295	31	326	9.5%
8–12	148	35	183	19%
14+	132	49	181	27%
Total	575	115	690	
				$P < 0.0001$

Table 4
Intraperitoneal (i.p.) hollow fibre activity versus xenograft activity in at least one-third of tested models where agent demonstrated activity in at least one xenograft

IP Score	% of Xenograft Models Active		Total	% Active in 33% of Xenografts
	<33%	⩾33%		
0-6	62	31	93	33%
8-12	32	35	67	52%
14+	43	49	92	53%
Total	137	115	252	
				$P = 0.011$

Table 5
Intraperitoneal (i.p.) hollow fibre activity versus xenograft activity in at least one model

IP score	Agents active in any xenograft	Agents inactive in all xenografts	Total	% Active
0–6	93	232	325	29%
8–12	67	116	183	37%
14–18	41	47	88	47%
20+	51	42	93	55%
Total	252	437	689	
				$P < 0.0001$

Table 6
Intraperitoneal (i.p.) hollow fibre activity versus i.p. xenograft activity

IP score	Agents active in IP xenograft	Agents inactive in IP xenograft	Total	% Active
0–6	35	219	254	14%
8–12	29	104	133	22%
14–18	22	44	66	33%
20+	25	40	65	38%
Total	111	407	518	
				$P < 0.0001$

reported that we had failed to find any correlation between the s.c. score in the hollow fibre assay and activity in xenograft models [4]. We have now determined though that for a specific range of i.p. scores, the s.c. score does help predict xenograft activity. We analysed 102 compounds with intermediate i.p. scores (between 14 and 20) and found that 58% of those compounds that also had s.c. scores of 8 or more had activity in at least one xenograft model, whereas only 35% of those with s.c. scores of 6 or less had activity in any xenograft models ($P = 0.016$, Table 7).

For compounds with an i.p. score of 0 or for those with an i.p. score of 22 or more, the s.c. score does not add value to the ability to predict for xenograft activity (data not shown). For those compounds that do not have any hollow fibre activity under conditions where drug is delivered i.p. to an i.p. tumour, we would hypothesise that activity in the s.c. fibres is not helpful in predicting xenograft activity as the activity seen in the s.c. fibres could plausibly represent non-specific toxicities. We also consider it reasonable that, for compounds with i.p. scores of 22 or more, the s.c. fibres do not add to our ability to predict efficacy in a xenograft model. As indicated in the preceding paragraph, in the intermediate range of i.p. scores (14–20), a s.c. score of 8 or more only provides us with a 58% probability of xenograft activity. In considering the i.p. score alone, as in Table 5, we already know that 55% of compounds with an i.p. score of at least 20 have activity in at least one xenograft model.

5. Question of disease type predictivity

As described above, hollow fibre panels representing specific histologies are used for specialised testing of compounds on a non-routine basis. Compounds are generally selected for testing in these histology-specific panels on the basis of selective activity against *in vitro* cell lines of the same histology or a biological rationale that suggests activity in a specific histology.

In the standard hollow fibre assay, only two cell lines of a given histology are used, so it is difficult to analyse potential correlations between hollow fibre activity in a given histology and xenograft activity in that same histology. However, the specialised hollow fibre panels described above do represent an opportunity to explore histology-specific correlations. For the melanoma, renal and prostate panels described above, there are as yet insufficient data to determine if there are meaningful correlations between activity in these panels and activity in xenograft models of corresponding histology.

However, for the leukaemia/lymphoma hollow fibre panel we have the minimum amount of data to begin to examine these issues. Potentially complicating issues include the fact that some of the data available are not from actual xenograft models, but from murine leukaemias/lymphomas in murine hosts. Additionally, under the umbrella of haematopoietic diseases, there are numerous biologically diverse diseases, and it may not be reasonable to assume that hollow fibre activity in the cell lines employed (see Table 1) can plausibly be expected to predict for activity in another haematological malignancy. Nevertheless, for those compounds where the i.p. score in the leukaemia/lymphoma fibres is 6 or more, keeping in mind that the maximum potential score is half that of the standard hollow fibre assay, 32% of compounds had activity in at least one traditional hematologic *in vivo* model. By contrast, only 11% of those with an i.p. score of between 0 and 4 had such activity (Fisher's exact, $P = 0.076$, Table 8). While we recognise that the P value is above the level normally set for statistical significance, this calculation was performed with the results of fewer than 50 compounds, only 10 of which had some level of subsequent *in vivo* activity, and we hope to repeat the analysis at a future date when more data are available.

6. Future directions

The key goal of using the hollow fibre model as a way to decrease the cost, both financial and in the number of animals used, to evaluate initial evidence of a compound's capacity to act across physiological barriers continues to be reinforced with our enlarging experience.

Table 7
Subcutaneous (s.c.) hollow fibre activity versus xenograft activity where i.p. hollow fibre score between 14 and 20

SC score	Agents active in any xenograft	Agents inactive in all xenografts	Total	% Active
0–6	17	32	49	35%
8+	31	22	53	58%
Total	48	54	102	
				$P = 0.016$

Table 8
IP score in leukaemia/lymphoma HF versus xenograft activity in leukaemia/lymphoma models

IP score leukaemia/ lymphoma HF	Agents active in any LL xenograft	Agents inactive in all LL xenograft	Total	% Active
0–4	3	24	27	11%
6+	7	15	22	32%
Total	10	39	49	
				$P = 0.076$

HF, hollow fibre; LL, leukaemia/lymphoma.

The hollow fibre methodology continues to allow the selection of agents with a higher likelihood of activity in classical xenograft models. Robust activity in a high proportion of models has classically predicted an increased likelihood of activity in clinical trials [4], at least for "cytotoxic" agents. In addition, preliminary extension of these efforts to histology-selective panels of cell lines suggests that this will be true within particular histological subsets of tumour cells. Whether this correspondence will continue in the era of "targeted" cancer therapy development remains to be determined, and will be influenced by, among other factors, how realistic the representation of the target in the cells used for the screening assays is as compared with the biological context of the disease.

The continued iteration of the hollow fibre technique will capitalise on the ability of the technique to inform at an early stage in a drug's evaluation upon the ability to affect particular molecular targets. This may even allow further refinement in lead selection through immunohistochemical, genomic, proteomic, and potentially enzyme assays of cells extruded from fibres removed from drug-treated animals. Pharmacological assay of fluid in fibres may allow an enhanced understanding of the drug's ability to diffuse into tissue spaces. The use of cells bearing imageable expression systems, such as the luciferase-based systems described elsewhere in this volume by Hollingshead and colleagues [9] may allow almost "real time" recording of drug effect within several minutes to hours after drug administration and therefore the speed and efficiency with which lead selection can proceed.

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