A014

Evaluation of the Hollow Fiber Model as a highly predictive in vivo testing system for the selection of xenograft tumor models in cancer drug discovery

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ProQinase TARGETING CANCER

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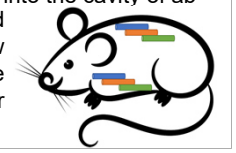
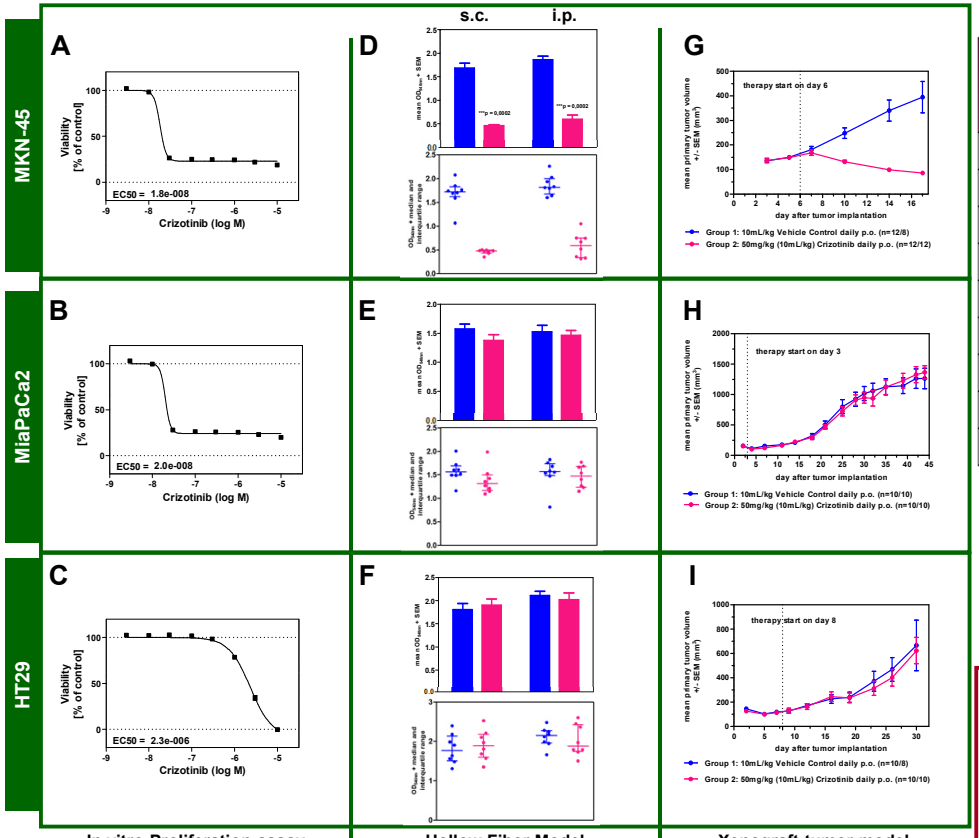
Introduction In cancer drug discovery, lead compounds showing anticancer efficacy in vitro need to be prioritized for further in vivo analysis. The Hollow Fiber Model* allows the evaluation of anticancer efficacy of a drug in three cell lines simultaneously in a single mouse. Following drug treatment, the number of viable cells in the fibers provides information about the drugs' potential in vivo anti-tumor efficacy.

To evaluate the predictive power of the Hollow Fiber Model we compared the antitumoral efficacy of the inhibitor Crizotinib on 9 tumor cell lines in the Hollow Fiber Model with the efficacy in a conventional xenograft study. The kinase inhibitor Crizotinib is a FDA-approved drug for NSCL, which inhibits the kinases ALK, MET and ROS1 and was run in a proliferation assay against a large panel of tumor cell lines to determine the IC50 values. Subsequently, 9 cell lines representing different inhibitor potencies via IC50 proliferation assay were chosen for comparison of the proliferation, Hollow Fiber Model and xenograft tumor model results.

*Hollingshead et al. (1995)

Method Six hollow fibers were loaded with three cell lines pairwise and implanted into two different body compartments of 8 NMRI nude mice: subcutaneously under the skin and intraperitoneally into the cavity of abdomen. After the mice were treated with Crizotinib for 14 days, hollow fibers were re-sected and the amount of viable cells in each fiber was quantified via MTT assay.





In vitro Proliferation assay

Proliferation assay using the CellTiter-Glo® Luminescent readout for MKN-45 (A), MiaPaCa2 (B) and HT29 (C). *Cells were incubated for 72 hours with Crizotinib followed by cell viability analysis.*

Hollow Fiber Model

Hollow Fiber Model in vivo using MKN-45 (D), MiaPaCa2 (E) and HT29 (F). *Mice were treated 14 days with Crizotinib (50mg/kg) and its Vehicle control daily orally. Content of tumor cells in hollow fibers subcutaneously (s.c.) and intraperitoneally (i.p.) were determined by MTT assay.*

Xenograft tumor model

Subcutaneous xenograft study example for MKN-45 (G), MiaPaCa2 (H) and HT29 (I). *Mice were treated with Crizotinib (50mg/kg) and its Vehicle control daily orally until study end. Tumor growth was monitored over time using caliper measurements.*

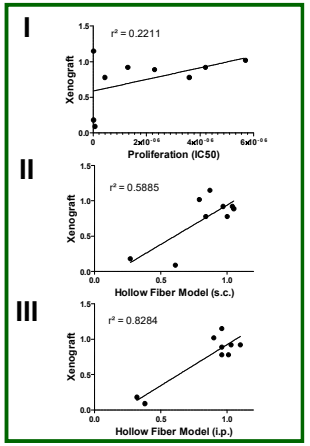
Summary

Cell line	Proliferation assay [IC50]		Hollow Fiber [T/c*] s.c.	Hollow Fiber [T/c*] i.p		Xenograft [T/c*]
MNK-45	1,8E-8	√ = √	0,27	0,32	√ = √	0,18
MiaPaCa2	2,0E-8	√ ≠ x	0,87	0,96	x = x	1,15
KARPAS299	7,0E-8	√ = √	0,61	0,38	√ = √	0,08
Molm-13	4,4E-7	√ ≠ x	0,84	1,01	x = x	0,78
Colo-205	1,3E-6	x = x	0,97	1,03	x = x	0,92
HT29	2,3E-6	x = x	1,05	0,96	x = x	0,89
HCT-116	3,6E-6	x = x	1,0	0,96	x = x	0,78
LN229	4,2E-6	x = x	1,04	1,1	x = x	0,92
SKOV-3	8,9E-6	x = x	0,79	0,90	x = x	1,02

* T/c= ratio of mean treatment / mean control

Xenograft outcome is well predicted by the Hollow Fiber Model, demonstrated by linear regression

=> 9 of 9 cell lines were correctly predicted by the Hollow Fiber Model, whereas only 7 of 9 cell lines were correctly predicted by proliferation assay.



Linear regression of proliferation assay IC50 (I), hollow fibers implanted s.c. (II) and i.p. (III) against xenograft in vivo studies.

Conclusions

- **In vivo Hollow Fiber Model as an intermediate step in the drug development process bridging the gap** between in vitro assay and xenograft in vivo studies (see cartoon on the right)
- **Considering in vivo pharmacodynamic and pharmacokinetic processes of test compound**
- **Animal reducement** according to the three rules of animal welfare (3R) **testing three cell lines simultaneously**
- A **defined and short assay time with low variability**
- **Predictive in vivo screening tool**