18th EORTC-NCI-AACR, Prague Nov 7-10 2006
Molecular Targets and Cancer Therapeutics
Abstract #314

contact:
Andrew Minchinton aim@bccrc.ca
Alastair Kyle akyle@bccrc.ca

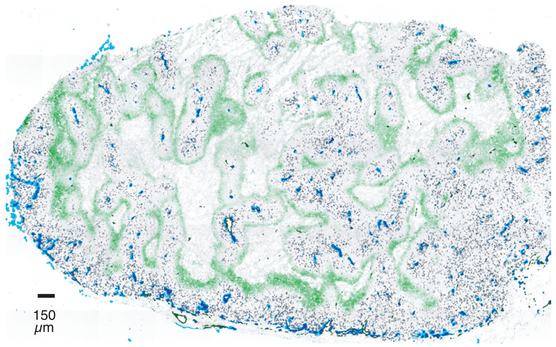
# Extravascular penetration of taxanes limit their efficacy

Alastair H. Kyle, Lynsey A. Huxham,
Devon M. Yeoman, & Andrew I. Minchinton

British Columbia
Cancer Research Centre
Vancouver, Canada

## Introduction
### *tumour drug penetration & in vitro screening*

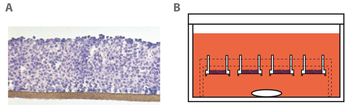
***Tumour biology***
Solid tumours contain a network of blood vessels supplying oxygen and nutrients. Tumour growth is limited by the balance struck between the consumption of nutrients & oxygen by the cells comprising the tumour and their supply from an expanding vasculature. Cells within tumours can be located up to ~15-20 cell layers (~150-250 µm) from a blood vessel, beyond which necrosis occurs.




staining key: blood vessels - dark blue (CD31 +ve)
blood flow -light blue (carbocyanine)
S-phase cells -black (BrdUrd)
hypoxic areas -green (Pimonidazole)

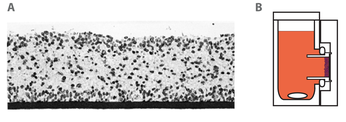
***Diffusion limited penetration***
The penetration of drugs into solid tumours is limited by the balance between drug diffusion and tissue consumption in a fashion similar to that of oxygen. Drug binding, metabolism and sequesterization within tissue all act to limit penetration.

***Multilayered cell culture***
Multilayered cell culture (MCC) is a three-dimensional tissue culture which mimics the tumour extravascular compartment and can be used to study drug penetration in a controlled environment.




H&E stained cryosection of a ~250 µm thick MCC grown on a permeable support membrane (**A**) and vessel used to grow cultures (**B**).

***Effect-based drug penetration assay***
In addition to direct visualisation of radiolabelled drug in MCC, their symmetrical growth allows drug penetration to be evaluated by comparing a drug's effect on the cells on either edge of the cultures following exposure to one side.



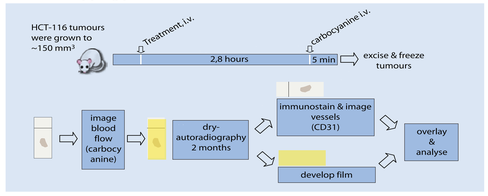

BrdUrd immunostained MCC showing symmetrical s-phase cell distribution (**A**). Apparatus used to perform drug penetration experiments (**B**).

## I - Tissue distribution of $^3$H-taxanes
### *tumour xenografts & multilayered cell culture*

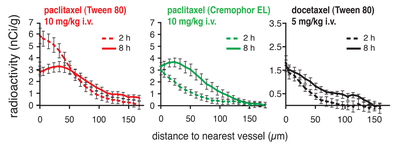
***Objective***: Map $^3$H-paclitaxel and $^3$H-docetaxel distribution in tumour xenografts in relation to vasculature and also in multilayered cell cultures (MCC), an *in vitro* model of the tumour extravascular compartment.

***Results:*** Both taxanes exhibited limited penetration, with little drug reaching further than 100 µm into tissue. Of the two, paclitaxel exhibited up to 2-fold greater penetration than docetaxel.

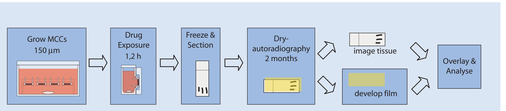
***Tumour mapping protocol***



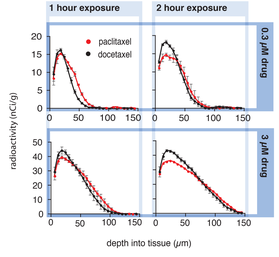

***Taxanes in HCT-116 xenografts:***
$^3$H-taxane distribution in relation to nearest tumour blood vessel



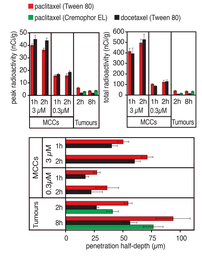

***MCC mapping protocol***




***$^3$H-Taxanes in HCT-116 MCCs:***




***Summary of xenograft & MCC-based results:***

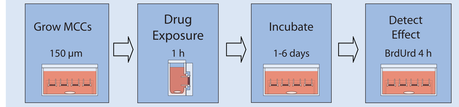



## II - Effect-based evaluation of drug penetration
### *multilayered cell culture based screening assay*

***Objective:*** Compare data from an *in vitro,* effect-based drug penetration screen with the radiolabelled taxane results.

***Results:*** The poor tissue penetration of the two taxanes was replicated in the effect-based assay where both exhibited limited effect when they had to penetrate into tissue to reach target cells. Paclitaxel again exhibited greater penetration than docetaxel.

***Effect-based drug penetration screening assay***

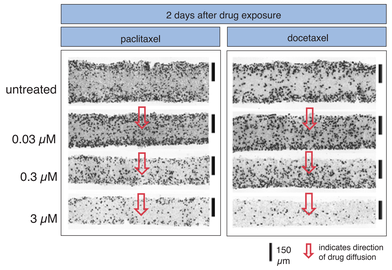



During growth the cultures have access to oxygen and nutrients from top and bottom and both edges exhibit high proliferation rates.
During drug exposure cultures are temporarily closed-off to allow drug to build up.

***Effect on S-phase cells within MCCs***

2 days after exposure to the taxanes from one side MCCs were immunostained for S-phase cells using BrdUrd.

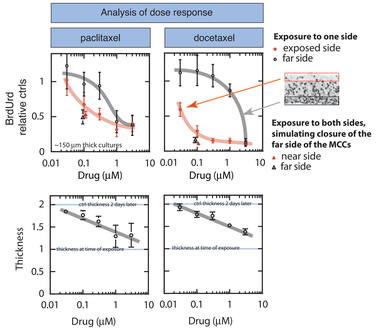
For docetaxel, cells on the unexposed side are significantly affected only after a 3 µM exposure.




***Analysis of drug effect on the exposed versus far side of MCCs***

Both drugs are seen to exhibit less effect on the far sides of cultures in relation the cells directly exposed to drug.

Analysis of effect-based data indicated that paclitaxel exhibits a more uniform effect on the two sides in comparison with docetaxel.

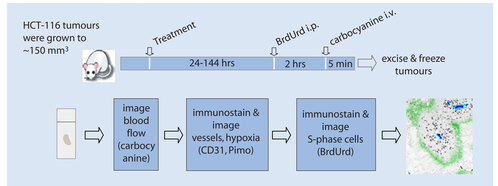



## III - Effect of taxanes in relation to vasculature
### *tumour xenograft mapping*

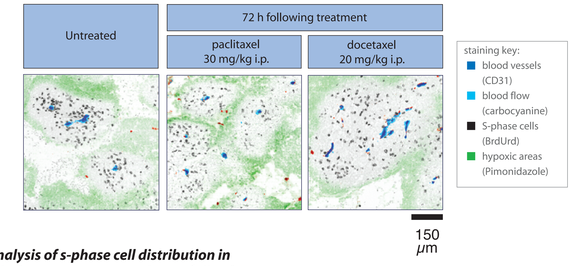
***Objective:*** Map distribution of s-phase cells in tumour xenografts in relation to vasculature following taxane treatment.

***Results:*** Interpretation of data is problematic due to the gradient in proliferation seen with depth into tissue. Data indicates both drugs were able to reduce proliferation by up to 75% near vessels but by only 50% in those cells distant from vasculature.

***S-phase cell mapping protocol***

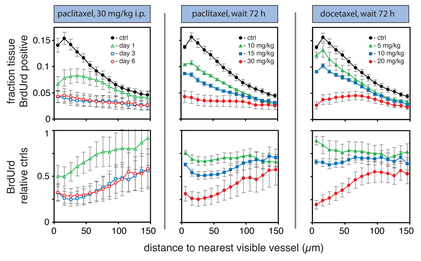



***S-phase cells 72 h following taxane treatment***




***Analysis of s-phase cell distribution in relation to tumour vasculature following taxane treatment***

Analysis of the effect of the drugs on proliferation as a function of distance away from blood vessels showed that their effects were greater in areas close to vasculature. Paclitaxel appeared to exhibit a greater effect in distant regions as compared to docetaxel.




## Acknowledgements
This work was funded by the Canadian Institutes of Health Research and the Breast Cancer Research Foundation, (BC & Yukon division). AHK is a Canadian Institutes of Health Research and Michael Smith foundation for Health Research Scholar. LAH is a Michael Smith foundation for Health Research Scholar.