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Targeting the tumour microenvironment: anti-vascular effects of the hypoxic cytotoxin tirapazamine

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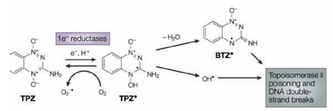
Background

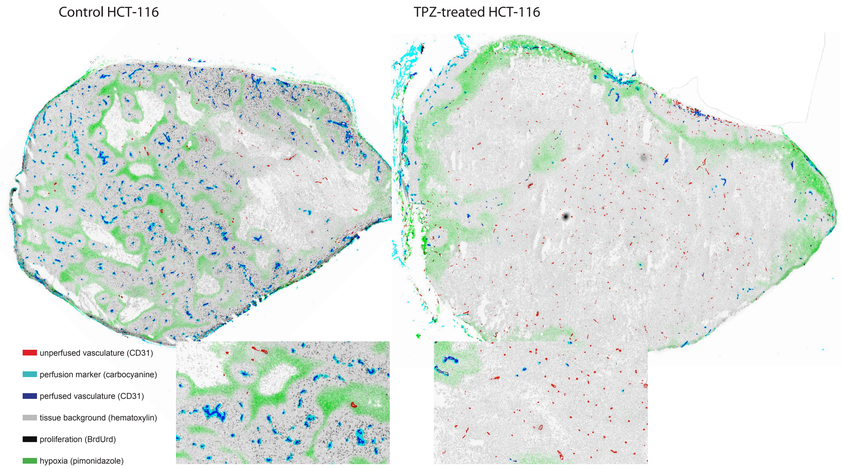
- **Tirapazamine** (TPZ) is a Phase III **bioreductive anti-cancer agent** with greater toxicity to hypoxic vs oxygenated cells *in vitro*.
- We have previously reported that TPZ is able to mediate dose-responsive, irreversible **central vascular dysfunction** *in vivo*, leaving a hallmark viable rim of surviving peripheral vessels as seen with other vascular targeting agents (VTAs).

{Huxham et al., 2004. Radiotherapy & Oncology (78) p138} {Huxham et al., 2008. Microvascular Research (75) p247}



{Brown & Wilson 2004. Nature Reviews Cancer (4) p437}

Tirapazamine-mediated vascular dysfunction

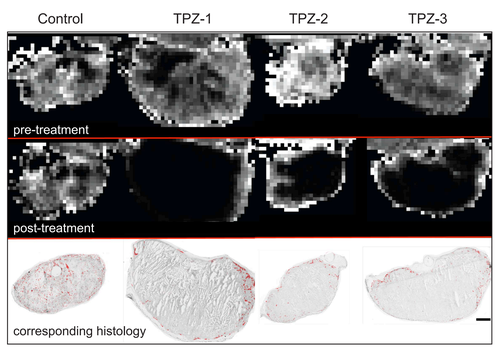


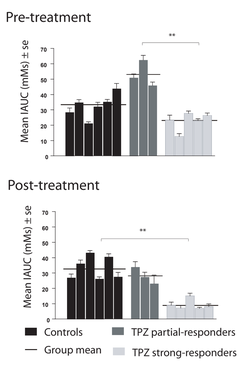
Tumour Mapping Analysis of Tirapazamine (TPZ)-Mediated Vascular Dysfunction

• The vascular targeting response to 60 mg/kg TPZ is characterized by a loss of perfusion in the central areas of tumours 12-24h after treatment, and is followed by onset of necrosis at 24-48h. HCT-116 colorectal xenografts exhibit a 65% response rate: 6-7 of 10 tumours exhibit central vascular dysfunction whereas 3 of 10 show no vascular response. A remaining rim of viable tissue is a hallmark of the effect.

• SCCVII murine carcinomas, Lewis Lung carcinomas and SiHa cervical xenografts are also sensitive to TPZ-mediated vascular dysfunction. HT-29 colorectal xenografts are resistant and do not show central loss of perfusion in response to TPZ.

• Tirapazamine-mediated vascular dysfunction may be detected using immunohistochemistry-based tumour mapping; multiple markers of the tumour microenvironment are labeled on the same section and overlayed for qualitative and quantitative analysis.





DCE-MRI Analysis of Tirapazamine-Mediated Vascular Dysfunction

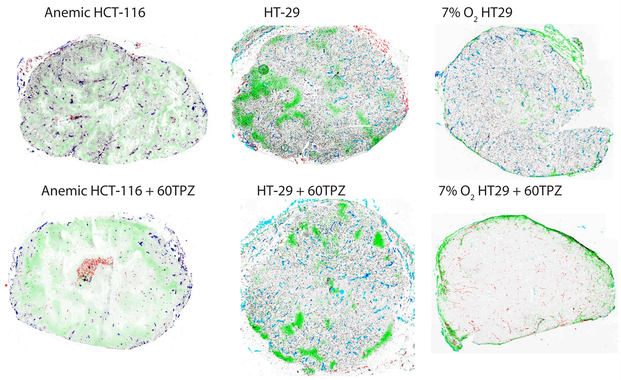
DCE-MRI analysis of Gd-DTPA uptake before and after treatment shows that the anti-vascular effects of tirapazamine may be observed non-invasively. HCT-116 tumours exhibiting greater pre-treatment perfusion were less likely to respond to the vascular targeting effects of TPZ and are classed as partial-responders.
Charts display average IAUC values for individual tumours before & after treatment with 60 mg/kg TPZ; **p<0.01.

Hypoxia

Poorly oxygenated tumours are more sensitive to TPZ-mediated vascular dysfunction.

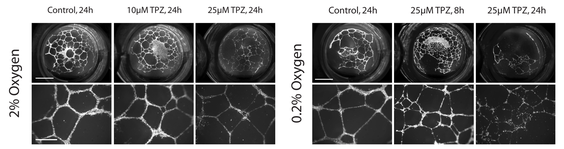
Anemic HCT-116 tumour bearing mice exhibit a greater magnitude and frequency of anti-vascular response to 60 mg/kg TPZ than in normal blood, air-breathing controls (left).

HT-29 tumours are typically resistant to the anti-vascular effects of TPZ (centre). However, HT-29 tumours exhibit a strong response when mice are exposed to lower oxygen (7%) during treatment (right).



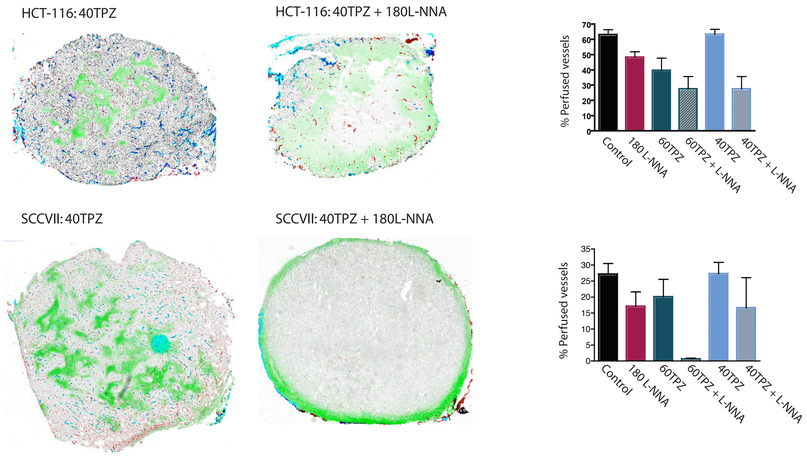
Human Microvascular Endothelial Cells (HMECs)

HMECS grown as tubes on Matrigel coated plates are sensitive to damage by TPZ in a concentration and oxygen dependent manner.



Nitric Oxide Synthase (NOS)

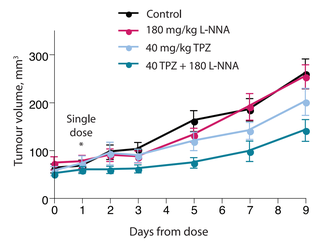
TPZ is reduced by cellular nitro-reductases to its activated form. Nitric Oxide Synthase (NOS) has a reducase domain that can activate TPZ; TPZ competitively inhibits nitric oxide synthase (NOS) under both hypoxic and oxygenated conditions {Garner et al., 1999. Cancer Research (59) p. 1929}. NOS isoforms are over-expressed in many tumours in addition to being expressed in tumour vasculature.



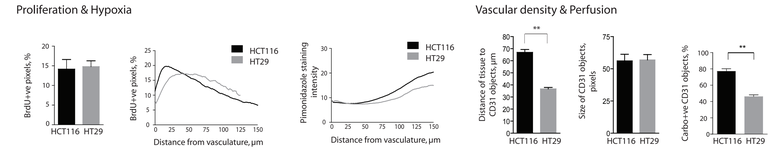
Combining TPZ with NOS inhibitor L-NNA potentiates the anti-vascular effects of TPZ occurring in the central regions of tumours.

SCCVII tumours typically exhibit a ~65% response rate to 60 mg/kg TPZ, with loss of perfusion at 24h and onset of necrosis by 48h. When combined with NOS inhibitor L-NNA the magnitude and rate of central vascular dysfunction response increases and the effect can be detected at lower doses of TPZ (40 mg/kg), when central loss of perfusion is rarely seen with TPZ alone. Doses of L-NNA alone cause loss of perfusion in vessels organized heterogeneously throughout the tumour, but rarely in a focused, central region of the tumour as is seen with other vascular targeting agents and TPZ.

HCT-116 xenografts also show an increase in response to both 40 & 60 mg/kg TPZ when combined with a NOS inhibitor, and this anti-vascular effect observed using tumour mapping studies (above) translates to inhibited tumour growth (right).

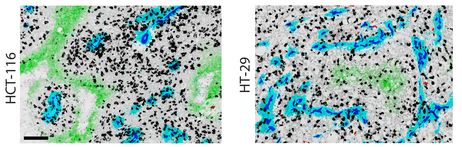


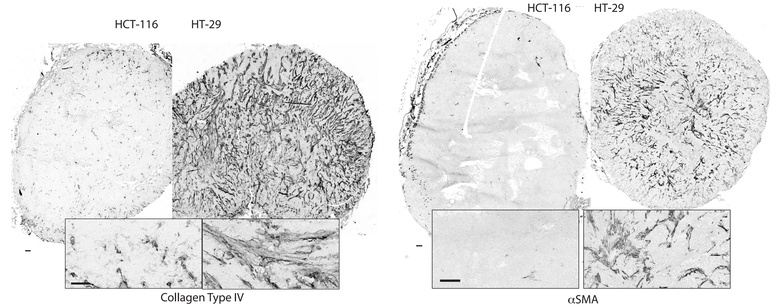
Vascular Phenotype



Comparing the tumour microenvironment of HCT-116 and HT-29 colorectal xenografts that are sensitive and resistant to TPZ-mediated vascular dysfunction respectively.

The microregional location of proliferating and hypoxic cells, tumour microvessel density and perfused blood vessel fractions vary between tumour models and may correlate with TPZ sensitivity.



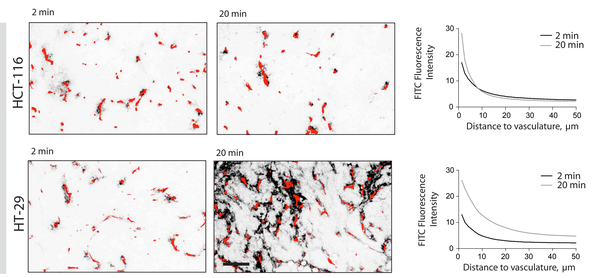


Comparing tumour blood vessel phenotype of HCT-116 and HT-29 colorectal xenografts that are sensitive and resistant to TPZ-mediated vascular dysfunction respectively.

A similar proportion of blood vessels are positive for collagen type IV in both HCT-116 and HT-29, however the layers are much thicker in HT-29 tumours. Similarly, HT-29 tumours have thicker layers of αSMA than HCT-116 tumours. However, fewer HCT-116 blood vessels are positive for αSMA than in HT-29, suggesting that HT-29 tumour vessels are more mature than those in HCT-116.

Tumour vascular permeability.

HCT-116 tumours are much are less permeable to extravasation or extravascular diffusion of high MW FITC-dextrans (black) from vasculature (red). The same molecule is able to extravasate and diffuse much further through the extravascular compartment of HT-29 tumours over time (right).



Summary

Tirapazamine (TPZ) is able to mediate catastrophic central vascular dysfunction in multiple murine and xenograft models *in vivo*, an effect that may be detected using tumour mapping or DCE-MRI. Using the rate of response in sensitive and resistant models as indicators of sensitivity, we have shown that anti-vascular effects of TPZ may be increased by varying the tumour oxygen conditions, suggesting that the mechanism for TPZ vascular dysfunction is related to its activity as a hypoxic cytotoxin. In addition, TPZ is reduced by the enzyme NOS, and we have shown that combining TPZ with NOS inhibitors potentiates both its anti-vascular and anti-cancer effects.
This work suggests that the tumour microenvironment confers tumour sensitivity to the anti-vascular effects of TPZ *in vivo* and emphasizes the importance of investigating the activity of anti-cancer agents in the context of the tumour microenvironment.

Acknowledgements

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