Microenvironmental distribution of trastuzumab is heterogeneous and decreases sharply when administered following a single dose of bevacizumab in Her2+ve xenografts and metastases models

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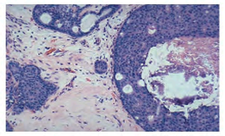
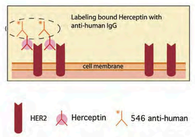
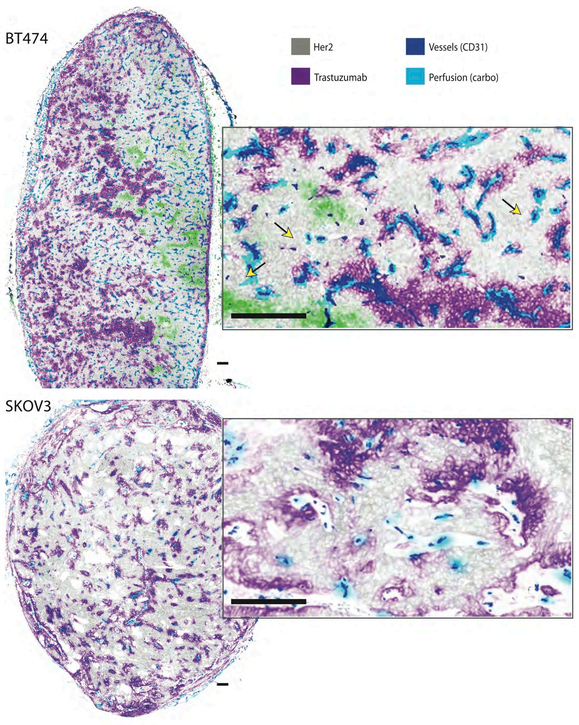
Background

- **Trastuzumab** (Herceptin) is a monoclonal antibody (MAb) approved for treatment of Her2+ve breast cancers.
- MAbs are relatively large (150 kDa; 6-10nm) therapeutics that may have difficulty distributing through tissue to access all target cells.
- Direct visualization of systemically administered, unlabeled trastuzumab within the context of additional features of the tumour microenvironment is done using anti-human fluorescent-tagged antibodies.



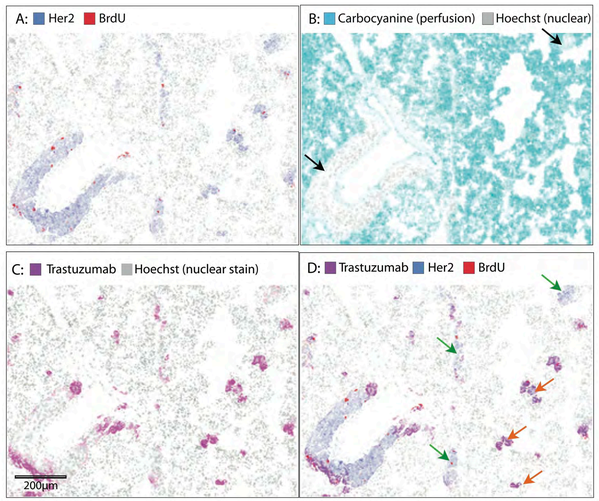


Heterogeneous distribution of Trastuzumab



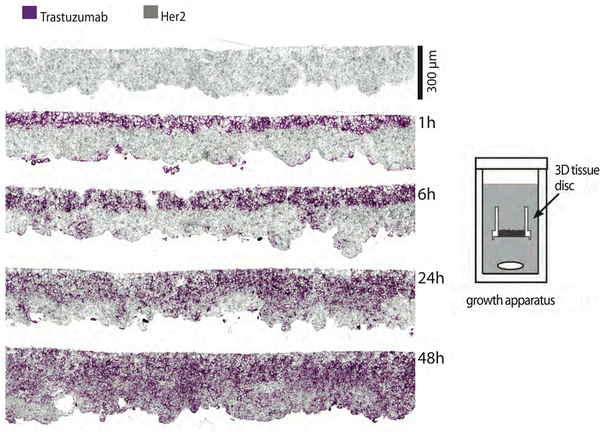
Signficant heterogeneity is seen in the microregional distribution of trastuzumab in Her2-overexpressing xenograft models of breast (BT474) and ovarian (SKOV3) tumours grown in NOD/SCID mice. Animals were treated with 10 mg/kg trastuzumab for 24h, with substantial amounts of Her2+ve tissue remaining unbound for trastuzumab. In the mouse model peak accumulation of MAbs occurs at 24-48h following administration; MAbs have longer plasma half lives in humans than mice, which would provide a longer exposure. However, the presence of perfused vessels that have an absence of perivascular bound trastuzumab suggests there is a barrier to trastuzumab distribution at the vessel or tissue level. SKOV3 tumours have greater amounts of perivascular stroma, where trastuzumab appears to have access to some tumour cells but cannot exit the stromal compartment in other areas. Scale bars 250μm.

Her2+ve metastases



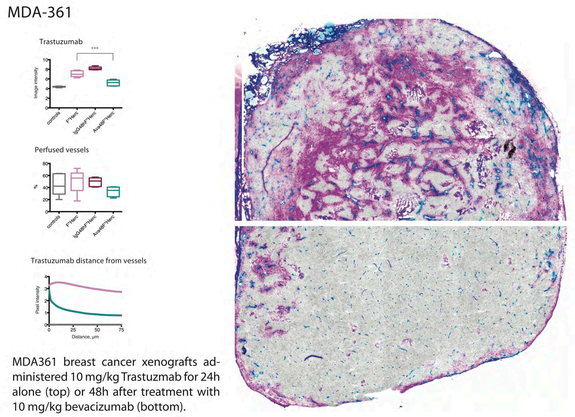
Limited access to trastuzumab in Her2+ metastases grown in mouse lung. Naturally Her2-overexpressing SKOV3 ovarian cancer cells injected into NOD/SCID mice grew as metastases ranging from just a few cells to larger nodules. Animals were administered 10 mg/kg trastuzumab and tissues were harvested at 24h. (A) Her2+ metastases in a range of sizes, all with a relatively low frequency of proliferating cells. (B) Lung tissues are highly perfused, however individual metastases have uneven exposure to the perfusion marker; black arrows indicate tumour tissue negative for carbocyanine. Exposure to trastuzumab was also limited in some metastases (C, D); orange arrows highlight example metastases with bound trastuzumab and green arrows indicate those with significantly less, despite the presence of Her2.

Distribution of Trastuzumab through 3D tissue discs

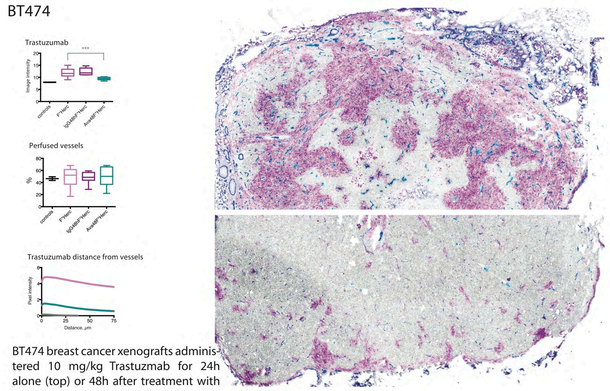


Her2-overexpressing MCF7 breast cancer cells grown as 3D tissue discs on permeable membranes were exposed to 50 μg/mL trastuzumab. Despite its large molecular weight and high binding affinity for Her2, trastuzumab reaches > 250 μm by 48h, which is greater than the average distance tumour cells reside from vasculature *in vivo*.

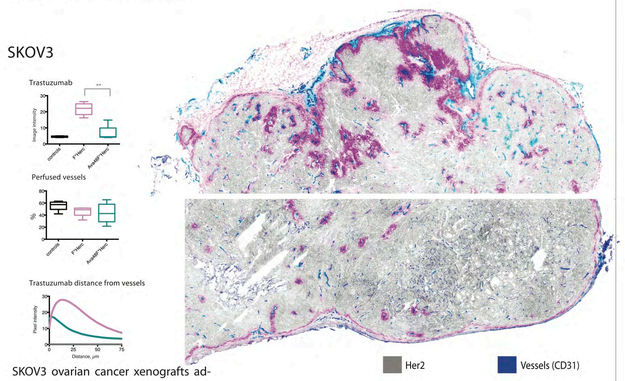
Bevacizumab pre-treatment decreases trastuzumab distributionn



MDA361 breast cancer xenografts administered 10 mg/kg Trastuzumab for 24h alone (top) or 48h after treatment with 10 mg/kg bevacizumab (bottom).

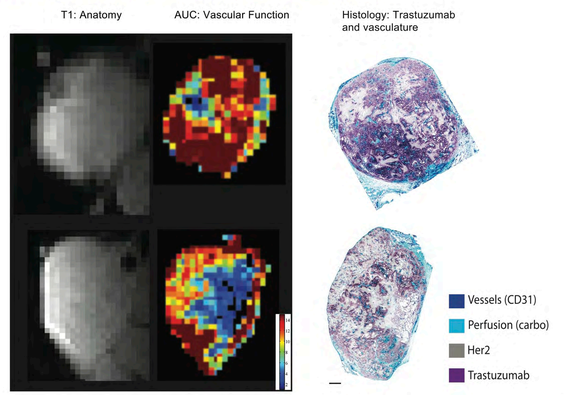


BT474 breast cancer xenografts administered 10 mg/kg Trastuzumab for 24h alone (top) or 48h after treatment with 10 mg/kg bevacizumab (bottom).



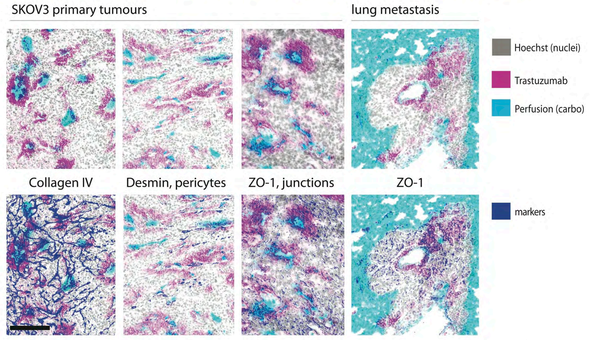
SKOV3 ovarian cancer xenografts administered 10 mg/kg Trastuzumab for 8h alone (top) or 48h after treatment with 10 mg/kg bevacizumab (bottom).

Impact of vascular function measured using MRI



Dynamic vascular function parameters (DCE-MRI) inconsistently reflect patterns of Trastuzumab distribution. A pattern of greater vascular function appears to reflect the pattern of Trastuzumab distribution, however this trend is not consistent Scale bars: 500μm.

Microenvironmental factors affecting Trastuzumab distribution



No correlation between basal lamina (CIV), pericytes (desmin), tight junctions (ZO-1), vascular density or vascular function and amount of bound trastuzumab. Staining for all of these markers exhibits inter- and intra-tumour heterogeneity that does not consistently correspond to areas of high or low trastuzumab accumulation. Scale bar 150μm.

Summary

- The extravascular distribution of trastuzumab in Her2-overexpressing primary xenografts and micro-mestastases is heterogeneous and is not explained simply by the presence or absence of vascular function.
- Despite its size and high binding affinity, trastuzumab distributes far through 3D tissue discs, suggesting the ability of trastuzumab to distribute via interstitial transport may not be compromised by the binding site barrier hypothesis.
- Pre-treatment with bevacizumab for even short exposures of 24-72h worsens the distribution of trastuzumab within the tumour microenvironment.

The microregional distribution of trastuzumab is highly heterogeneous and appears to be independent of conventionally considered barriers to drug delivery. Persistence of tissues unbound for trastuzumab suggests the possibility of inadequate drug exposure as a mechanism for drug resistance that is exacerbated by pre-treatment with VEGF blockade.

Acknowledgements

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