www.sciencetranslationalmedicine.org/cgi/content/full/7/284/284ra57/DC1

# Supplementary Materials for

## An implantable microdevice to perform high-throughput in vivo drug sensitivity testing in tumors

Oliver Jonas, Heather M. Landry, Jason E. Fuller, John T. Santini Jr., Jose Baselga, Robert I. Tepper, Michael J. Cima, Robert Langer*

*Corresponding author. E-mail: rlanger@mit.edu

Published 22 April 2015, *Sci. Transl. Med.* **7**, 284ra57 (2015)
DOI: 10.1126/scitranslmed.3010564

**The PDF file includes:**

# MATERIALS & METHODS:

### Cell lines and tissue culture conditions

Human cancer cell lines PC-3 (prostate), BT474 (breast), and A375 (melanoma) were obtained from American Type Culture Collection (ATCC). A-375 and PC3 lines were cultured in Dulbecco's Modified Eagles Medium (DMEM) (Invitrogen) with 10% fetal bovine serum (FBS). BT-474 was cultured in DMEM-Nutrient Mixture F-12 (Invitrogen) with 10% FBS. Cells were maintained in conditions of 37°C and 5% $CO_2$ and passaged 1-3 times per week upon reaching ~70% confluence. Cells were used at passage numbers 5-20. All cell lines used were tested for mycoplasma contamination.

### Mice and xenograft tumors

Tumor cells (~$3\times10^6$) in 150 μL PBS were injected subcutaneously into the flanks above the hind limbs of 6-8-week-old female athymic nude mice (Crl:NU(NCr)-Foxn1$^{nu}$) purchased from Charles River Laboratories. Tumors grew for approximately 3-4 weeks, to a size of 5-9 mm, before device implantation. Animals were chosen randomly for experiments when tumor size was between 5 and 9 mm. No specific method was chosen for randomization. The triple-negative breast cancer model was grown by subcutaneously implanting tumors in 6-week old athymic nude mice. Upon xenograft growth, tumor tissue was reimplanted into recipient mice. After each experiment (between 4 to 48 hours), mice were euthanized in order to collect the tumors. In some cases, mouse tumors were biopsied with a 13-G Cassi biopsy gun (Scion) to retrieve the tissue immediately surrounding the device. Whole tumors containing the device inside were either fixed for 24 hours in 4% paraformaldehyde for paraffin embedding or frozen in optimal cutting temperature gel and stored in -80°C for frozen sectioning. All animal studies were conducted in accordance with protocols approved by the Committee on Animal Care (CAC) at the Massachusetts Institute of Technology.

### Immunohistochemistry (IHC)

Tumor-device specimens were fixed in 4% paraformaldehyde for 24 h and subsequently processed and embedded in paraffin for IHC. Tissue section staining for apoptosis and proliferation-specific markers was performed by the Koch Institute Histology Core Facility. Rabbit monoclonal antibodies directed against human cleaved caspase 3 (Cell Signaling Technologies, #9664, diluted

1:150), human cleaved PARP (Cell Signaling Technologies, #5625, diluted 1:200), human Survivin (Cell Signaling Technologies, #2808, diluted 1:150), and a mouse monoclonal antibody directed against human Ki67 (Vector Laboratories, VP-K452, diluted 1:150) were used as primary antibodies. Primary antibody binding was detected using DAB staining with hematoxylin as a counterstain. Stained cells were captured using the EVOS XL Cell Imaging System (Advanced Microscopy Group).

**Scoring of IHC sections:**

Percent area of DAB stained cells was calculated for 100 μm × 100 μm regions in tissue directly perpendicular to the reservoirs. The analysis was performed in a semi-automated fashion using ImageJ Color Deconvolution with the vector "H DAB". A threshold was set to eliminate background staining and all outliers smaller than 2 pixels in diameter were removed. The Smooth function was also used to fill in cells that did not have completely homogenous staining. Error bars represent one standard deviation. In a small number of samples (<10%), device reservoir was located in a region of tumor that was highly necrotic. These sections were not included in the analysis because the differential effect caused by the presence of drug in this tissue region cannot be determined independently.

# SUPPLEMENTARY FIGURES:

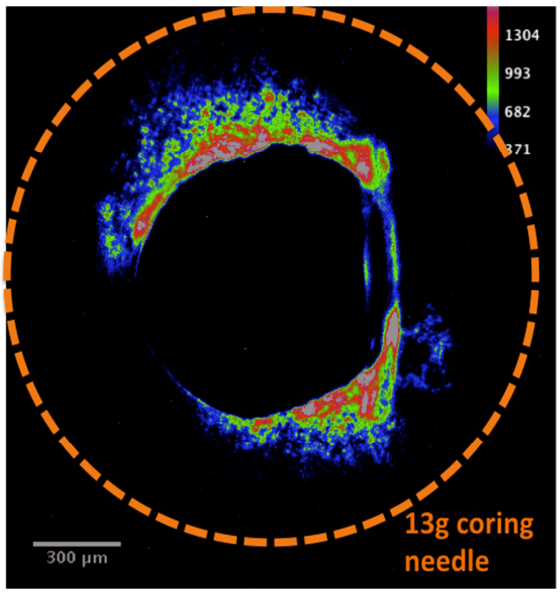


**Figure S1. Removal of drug-exposed tumor region by a coring needle.** The device and the region of drug distribution was removed by a 13-gauge coring needle after 20-24 h for pure and formulated drug contents. More than 99% of drug was removed, as measured by autofluorescence of pure doxorubicin.

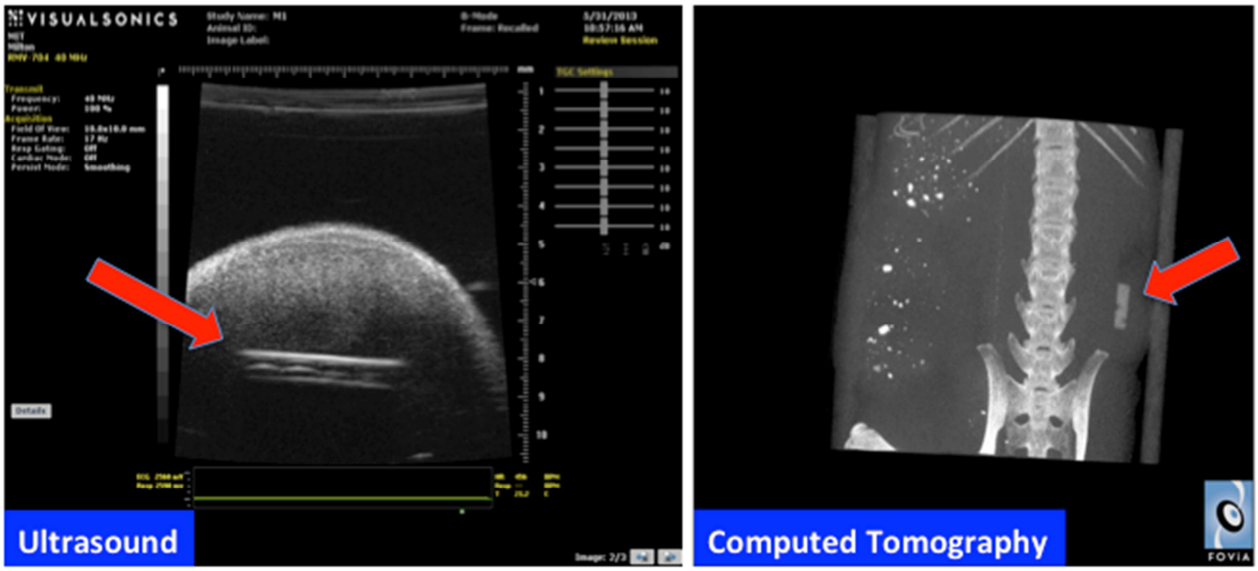


**Figure S2: Visibility of device for in vivo imaging with ultrasound and CT.** Ultrasound was used to image a device implanted 1 cm into a subcutaneous human BT474 xenografted tumor in a mouse. Computed tomography (CT) was used to image a device implanted in 1 cm orthotopic human MDA-MB-231 breast tumor in a mouse.

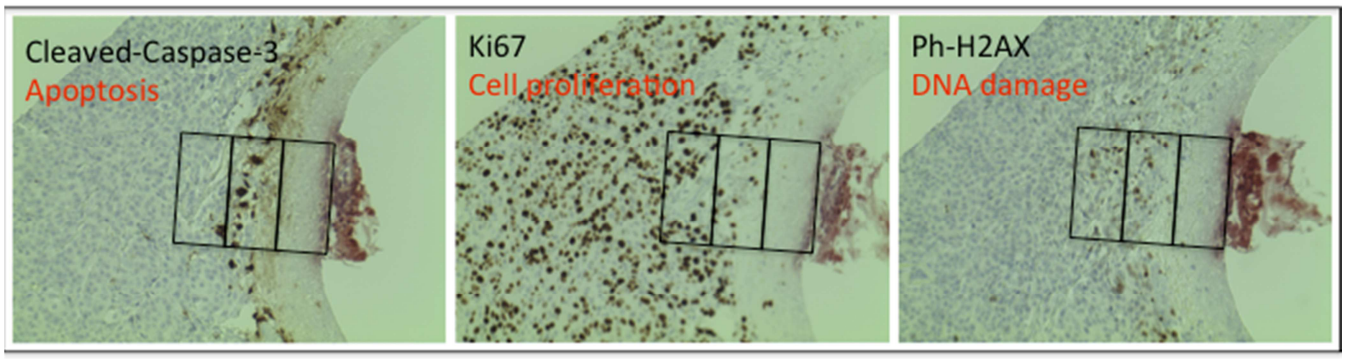


**Figure S3: Device/tissue cross sections stained for multiple drug sensitivity markers**. IHC was performed in directly adjacent 5-μm sections, showing time- and concentration-dependent effects on apoptosis (cleaved caspase 3), proliferation (Ki67), and nuclear damage (Ph-H2AX) of cells in human BT474 tumors in mice, exposed to 100% pure doxorubicin for 18 h. Each box width represents 100 μm.

**Table S1: Solvents for and concentrations of drug-polymer mixtures for device reservoirs.**

| Drug | Solvent (evaporated) | Drug ratio in PEG (%) |
|---|---|---|
| Doxorubicin | None | 20 in Fig. 6; otherwise 40, 5, 1, or 100% pure drug (as described for each experiment) |
| Paclitaxel | Acetone | 40 |
| Gemcitabine | None | 60 |
| Cisplatin | Deionized (di) $H_2O$ | 40 |
| Lapatinib | Acetone | 80 |
| Topotecan | di$H_2O$ | 30 |
| Vemurafenib | Acetone | 50 |
| Sunitinib | Acetone | 50 |