IECC 2021
The 1st International Electronic Conference on Cancers
Exploiting Cancer Vulnerability by Targeting the DNA Damage Response
01–14 FEBRUARY 2021 | ONLINE

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Anti-inflammatory combinatorial therapy to enhance killing efficacy with patient-derived preclinical models

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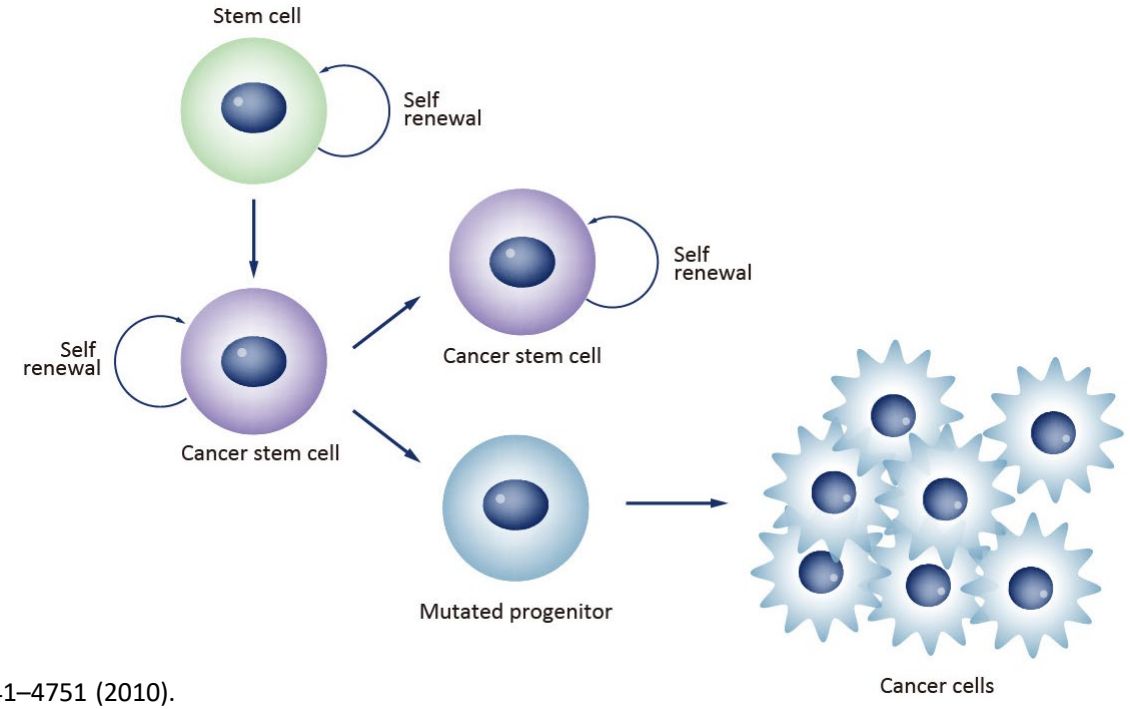
Cancer stem cells (CSCs)

- **Drug-resistant** cancer phenotypes is a challenge for anti-cancer therapy.
- CSCs are relatively quiescent cells capable of **self-renewal**.
- CSCs are identified as one of the ways by which chemoresistance develops.



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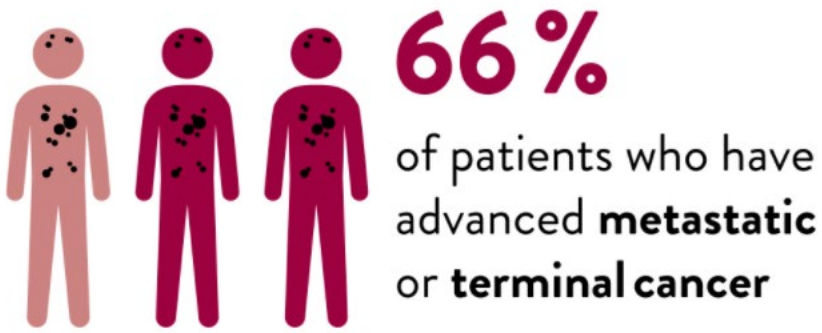
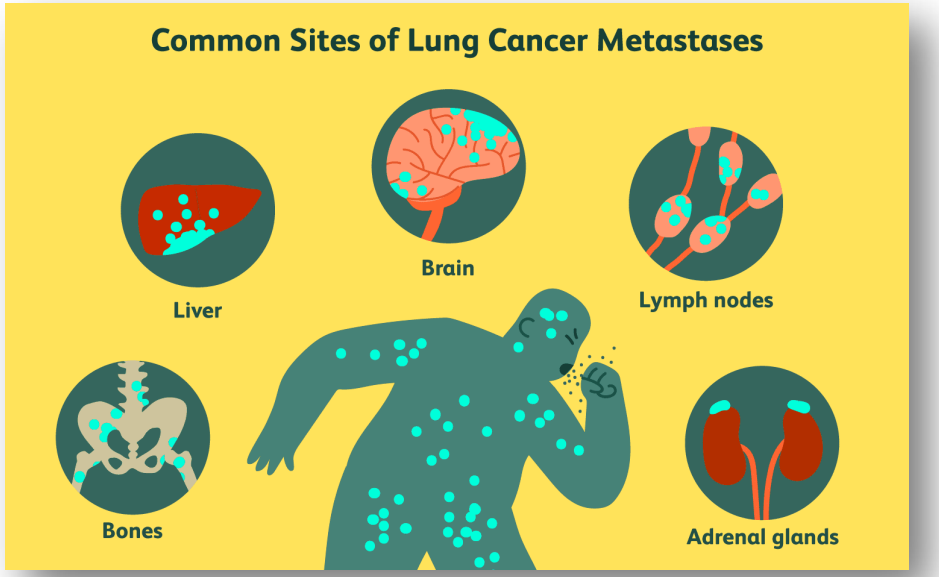
Cancer metastasis

- Cancer **metastasis** is the major cause of cancer morbidity and mortality
- accounts for about **90%** of cancer **deaths**
- a process in which cancer cells disseminate **from** the **primary** tumor, **settle and grow** at a site other than the primary tumor site.





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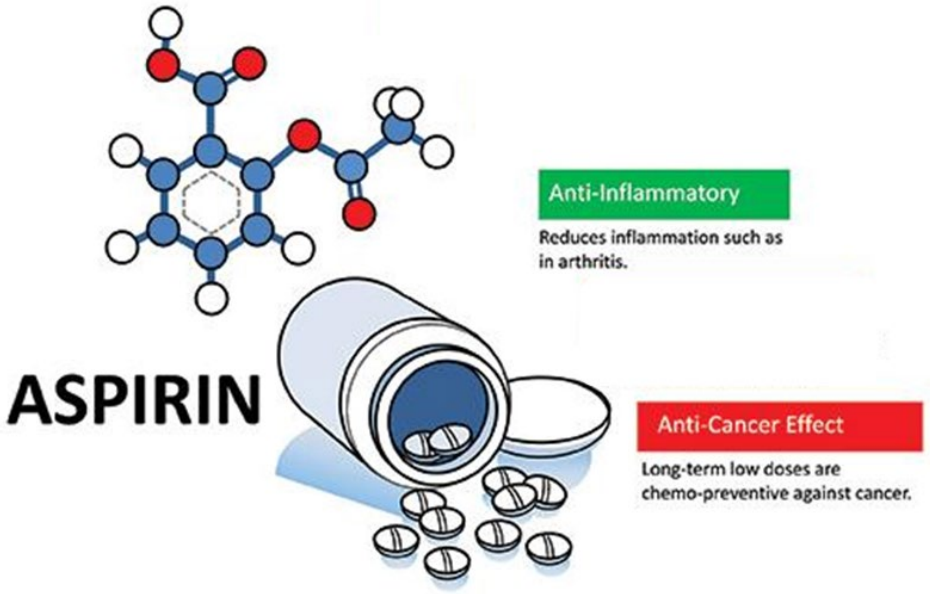
COX-2 and cancer

- An inhibition of redox-responsive cyclooxygenase (COX) enzymes is often attributed to the mechanism of **aspirin**.
- COX-2 regulates tumour **growth**, **invasion** and **metastasis** in breast cancer.
- the pro-neoplastic effects of COX-2 action



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ROS and DNA damage

- Reactive oxygen species (ROS) are a group of short-lived, highly reactive, oxygen-containing molecules
- induce **DNA damage** and affect the DNA damage response (**DDR**).
- **COX-2** expression could be triggered by **ROS**
- ROS favors the expression of an inflammatory phenotype that leads to the **induction** of COX-2.

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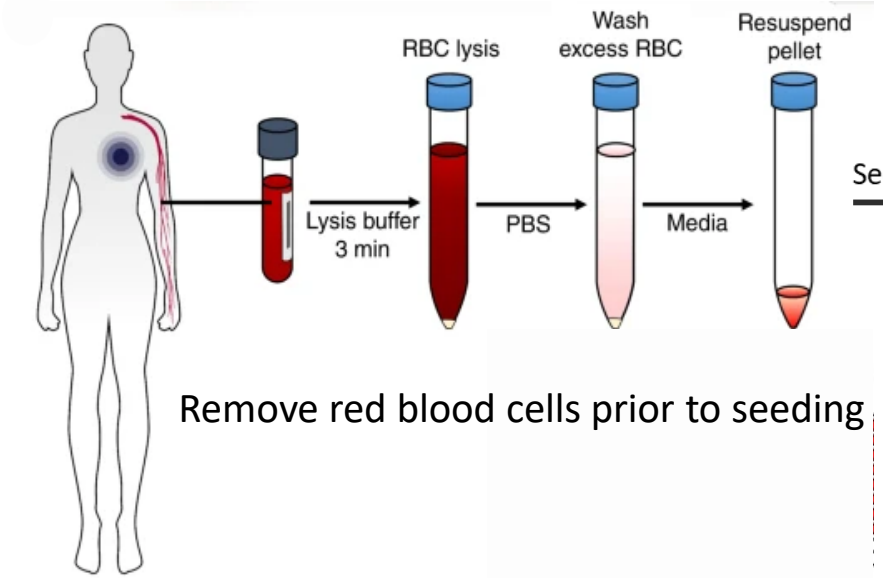
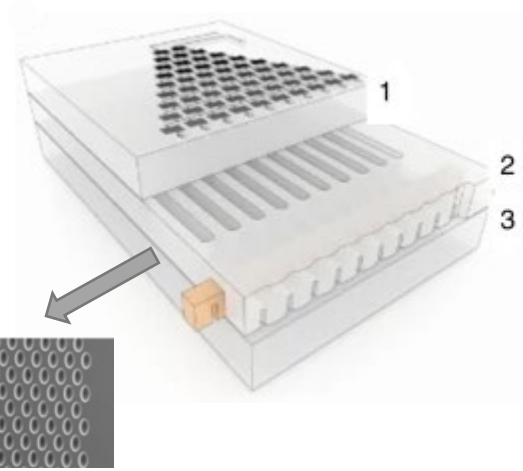
Circulating tumor cells (CTCs) cluster assay

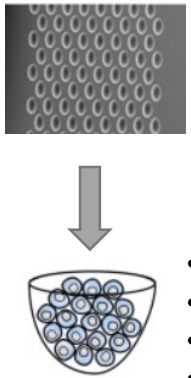
- CTCs are a rare subset of cells found in the blood of patients with solid tumors.
- We demonstrate an efficient approach to evaluate drug response using patient-derived CTC cultures obtained from liquid biopsy.



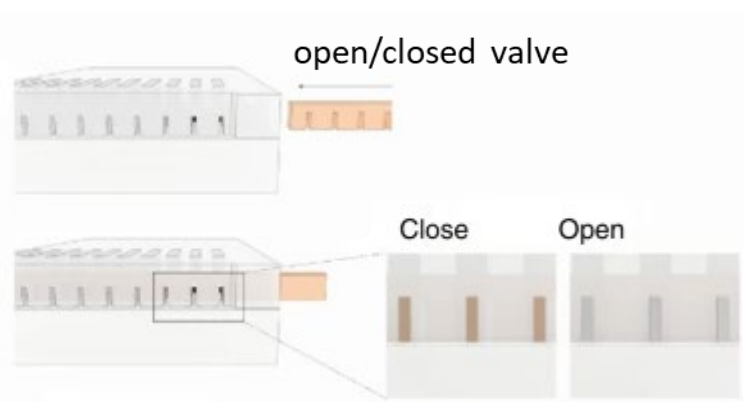
Remove red blood cells prior to seeding

Seed cells





- microenvironmental mimic
- three-dimensional
- multilayered
- under low shear

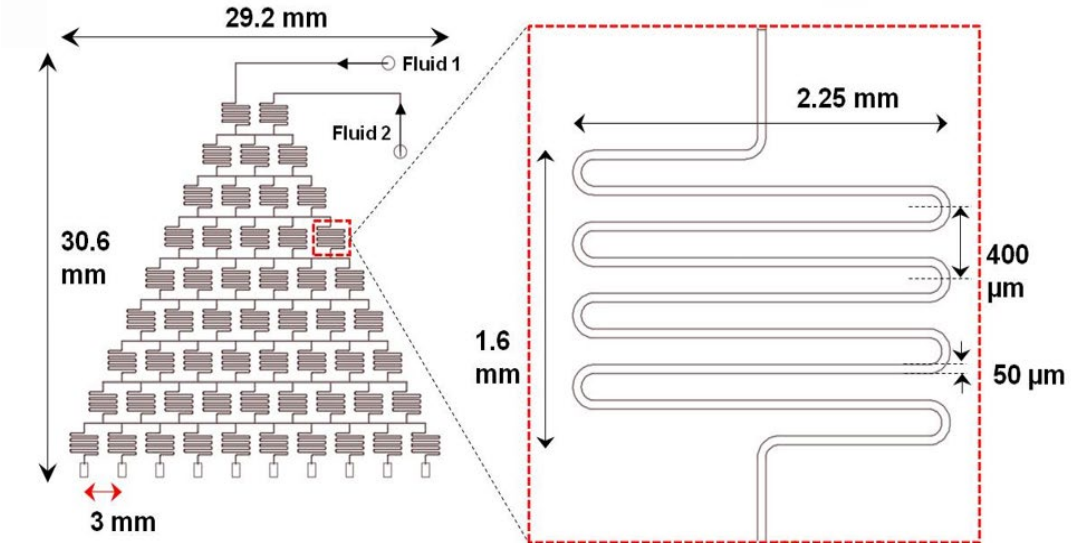


1: a gradient generator
2: open channels of the middle layer
3: custom-designed tapered microwells

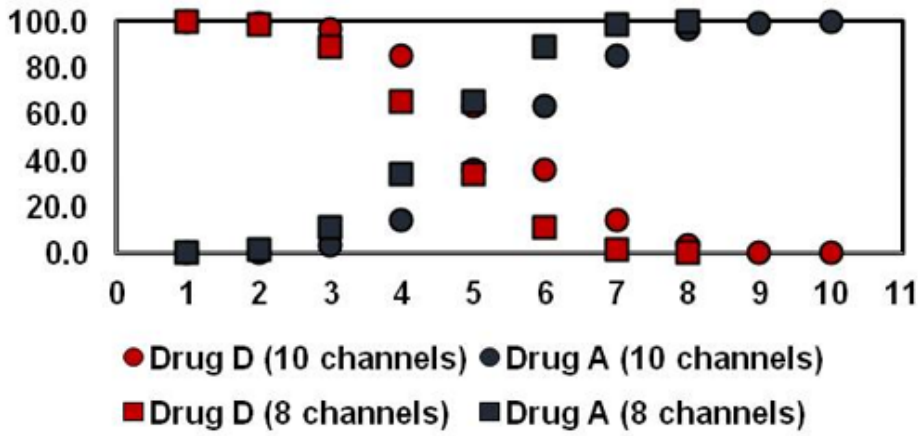
Yang, C., Xia, B. R., Jin, W. L., & Lou, G. (2019). Cancer Cell International, 19(1), 341.
Khoo, B. L. et al. Sci. Adv. 2, e1600274 (2016).
Khoo B L, Grenci G, Lim J S Y, et al. 2019, 120(4): 407.

Combinatorial therapy

DA treatment: In this proof of concept study, a range of therapeutic drug concentrations for 0–500 mg/ml aspirin (A) and 0–1 µM doxorubicin (D) were screened with a microfluidic culture and drug-screening assay validated for primary cell cultures.



Schematics of the gradient generator



Distribution of two drugs in combination with the effect of gradient generator

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Significance of liquid biopsy and CTCs

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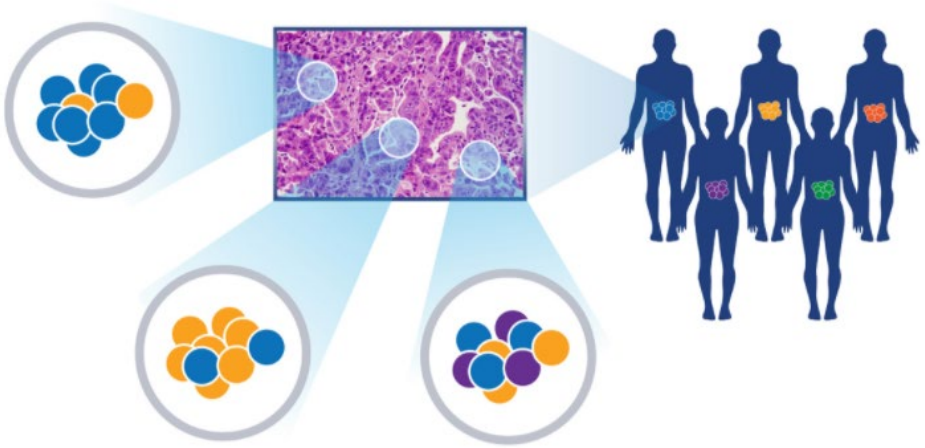
- Cancer is a **dynamic** disease. During the course of disease, cancers generally become more **heterogeneous**.
- **Heterogeneous response** of therapy highlights the importance of **patient-derived** preclinical models.
- lack of a robust anticancer **drug screening** system to monitor patients during treatment.
- Our CTC assay obtained from liquid biopsy is **efficient**, **non-invasive**, **inexpensive** and **drug screening** for personalized treatment.

Methods	Limitation
Imaging techniques	false-negative findings
Tumor biopsies	invasive, cancer gene reflecting incompletely
Multiwell plates	multiple passages, phenotypes changes
Other CTC expansions	pre-enrichment required, low efficiency



Heterogeneity

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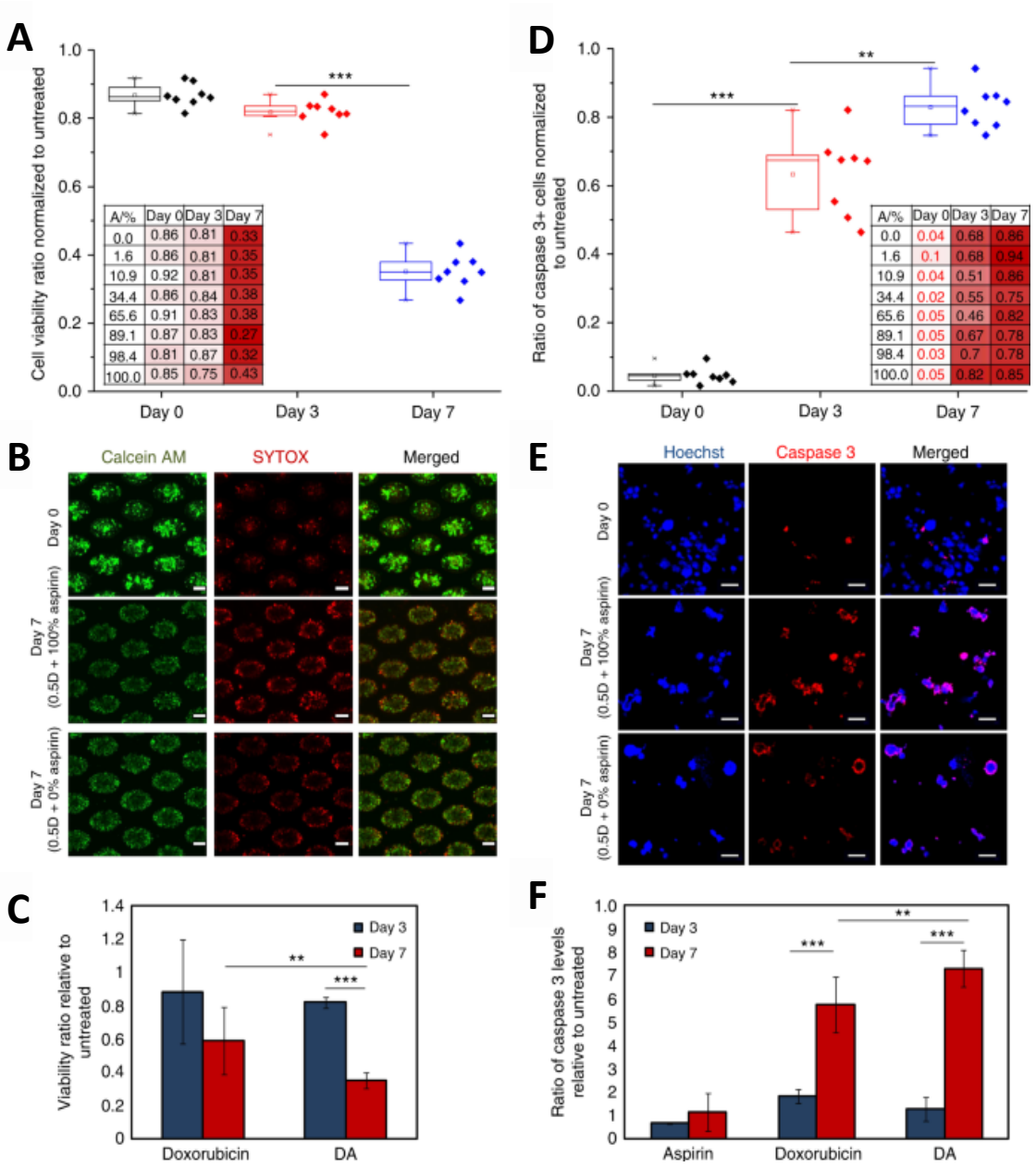
DA therapy improved killing efficacy and apoptosis



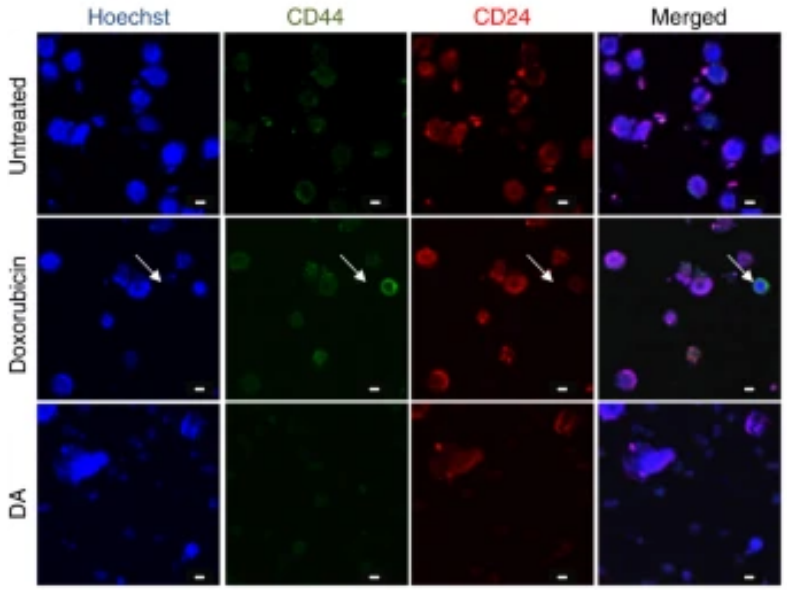
A/%	Day 0	Day 3	Day 7
0.0	0.86	0.81	0.33
1.6	0.86	0.81	0.35
10.9	0.92	0.81	0.35
34.4	0.86	0.84	0.38
65.6	0.91	0.83	0.38
89.1	0.87	0.83	0.27
98.4	0.81	0.87	0.32
100.0	0.85	0.75	0.43

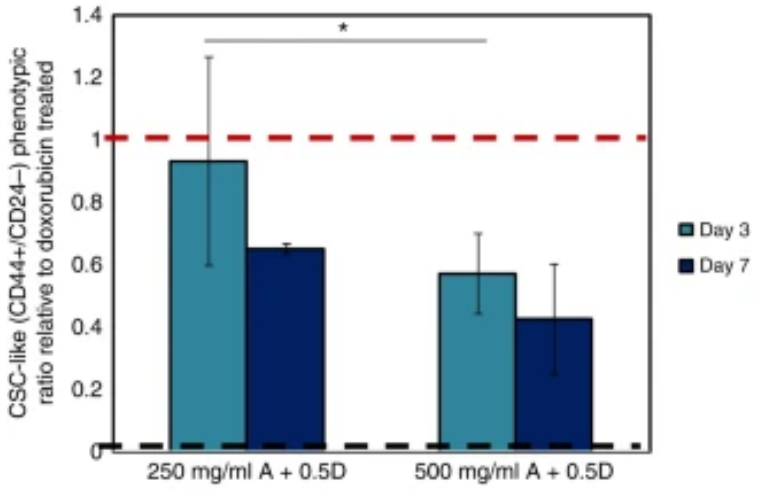
A/%	Day 0	Day 3	Day 7
0.0	0.04	0.68	0.86
1.6	0.1	0.68	0.94
10.9	0.04	0.51	0.86
34.4	0.02	0.55	0.75
65.6	0.05	0.46	0.82
89.1	0.05	0.67	0.78
98.4	0.03	0.7	0.78
100.0	0.05	0.82	0.85

- **Viability** ratio breast cancer cell line MDA-MB-231 cultures decreases after prolonged exposure to combinatorial DA treatment.
- **Killing efficacy** of DA treatment surpassed that of treatment with doxorubicin **alone** after 7 days of exposure, as determined by the live and apoptotic cell proportions.

Khoo B L, Grenci G, Lim J S Y, et al. 2019, 120(4): 407.

DA therapy reduced cancer stem cells

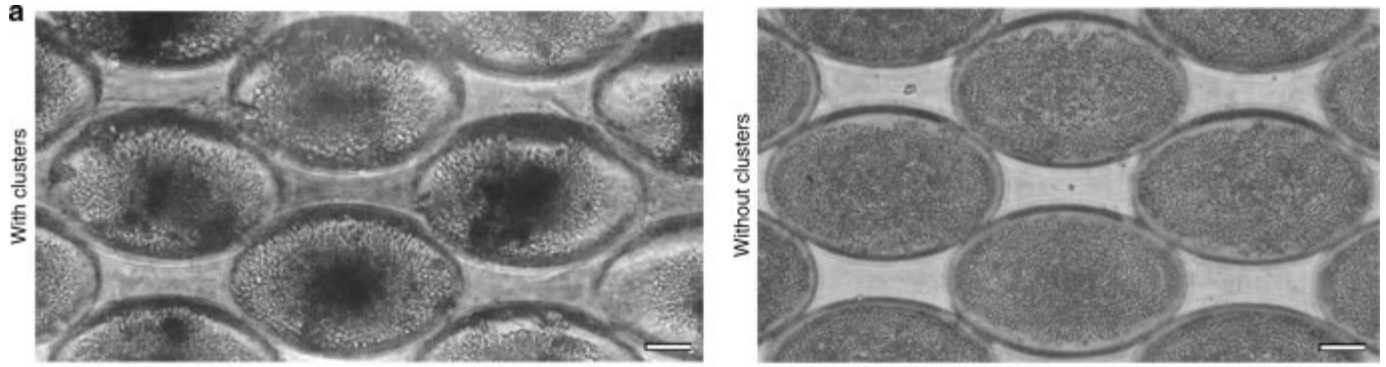


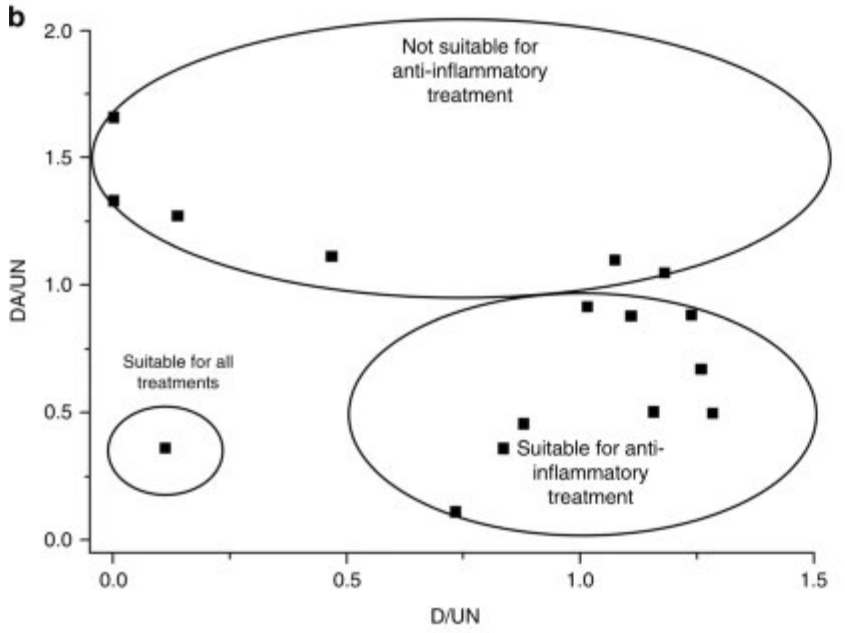


- **Increase in CSCs** post treatment have a high potential to form secondary **metastases**, leading to cancer relapse.
- $CD44^{+}/CD24^{-}$ phenotype corresponded to the proportion of **CSC-like** cells
- DA treatment with **500 mg/ml** aspirin resulted in a significant **reduction of CSC proportion.**

DA therapy in clinical cohorts

- Representative images of microwell array with **clusters** (left) and without clusters (right). Scale bar is 50 µm.
- Patient samples that responded in terms of killing efficacy are marked in red.
- Heterogeneity of patient profiles and the reliable patient-derived models are importance for screening similar anti-inflammatory and anti-cancer strategies





c

Suitable for anti-inflammatory treatment		Not suitable for anti-inflammatory treatment	
ASL32	0.39056	CES105	1.02654
BC234	0.15439	CES093_2	1.33
BC249	0.43289	CES76	1.66
BC241	0.43708	ASL36	2.39023
BC239	0.52311	CES102	3.27325
CES64	0.71535	CES093_1	9.2865
CES78	0.78291		
CES16	0.79557		
CES83	0.89144		
BC240	0.90546		

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DA treatment acts via a COX-independent pathway mediated by anti-inflammatory mechanisms

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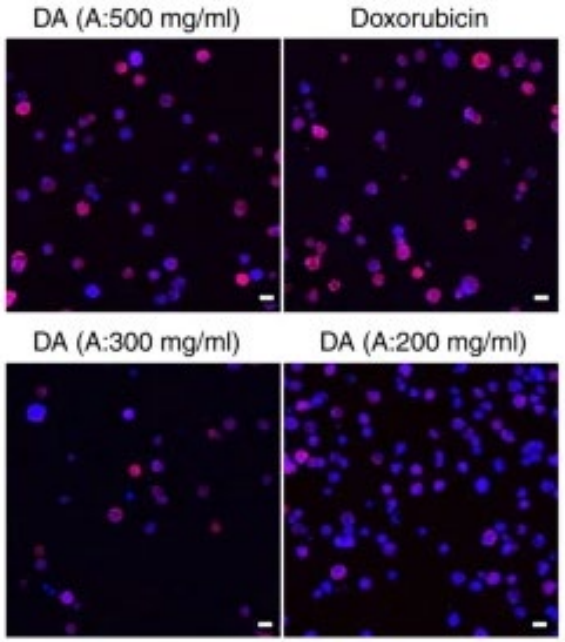
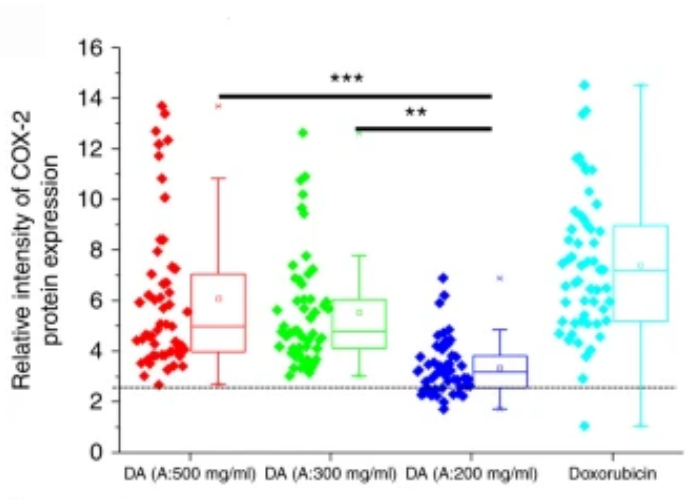
- COX-2 protein expression was **reduced** under combinatorial DA treatment.
- COX-2 reduction is directly associated with **malignancy and resistance**.
- Reduction in COX-2 protein expression was significantly greater under low-dose aspirin (200 mg/ml).
- DA treatment is more **effective** in its anti-cancer effects.



Immunostaining for COX-2 protein.
Scale bar is 10 µm.



Relative intensity levels of COX-2 protein after 7 days of drug exposure.

DA: combinatorial treatment with 0.5 D and 500 mg/ml aspirin.
Doxorubicin treatment: treatment with 0.5 µM dosage of doxorubicin

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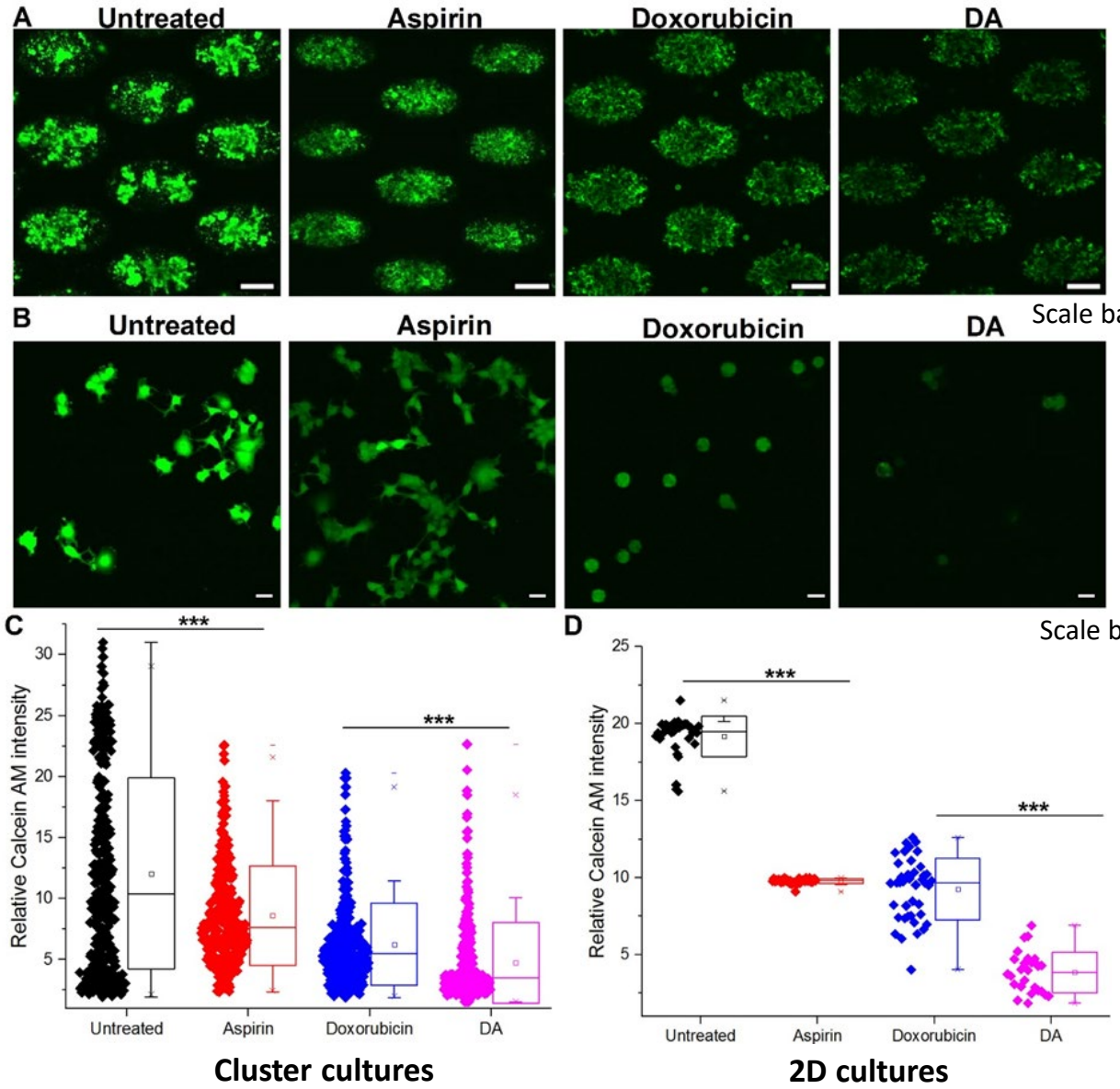
DA treatment reduces intracellular oxidative activity

- Calcein AM is also an indicator of intracellular oxidative activity.
- The differential intensity of Calcein AM suggests a **reduction** in **intracellular oxidative activity** in samples under both single drug aspirin and combinatorial DA therapy.
- Intracellular oxidative activity is a factor in metabolism and a key regulating process for several core functions including **cell proliferation** and **transcription**.



Cluster cultures **2D cultures**

Uggeri, J. et al. Cell Biol. 122, 499–505 (2004)

DA treatment revert cells to a less metastatic phenotype

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- **Increased ROS levels** causes cancer development and **metastasis** during or after chemotherapy.
- Extracellular **ROS** help to facilitate the formation of multifoci tumors and act as a springboard for **metastatic tumor cells**.
- Cancer cells usually present as a hybrid phenotype and express intermediate levels of **epithelial (E)** and **mesenchymal (M)** characteristics.
- With DA treatment, the proportion of cells with **epithelial-like phenotypes** were **increased** within 72 h and more apparent after 7 days.

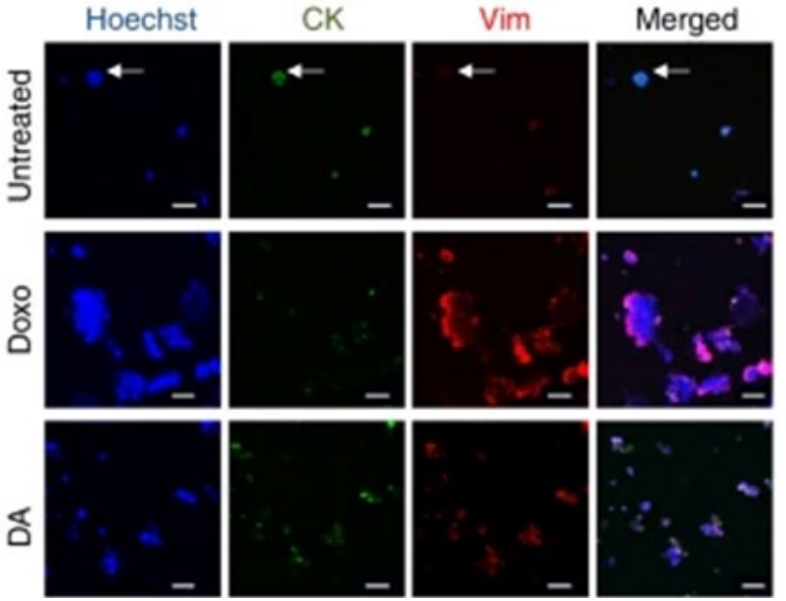
Aspirin concentration

Day 3	
Epithelial-like	Intermediate
0.80	0.99
0.57	1.22
1.07	0.71
2.14	0.37
3.16	0.38
1.43	0.60
1.40	0.81
1.00	1.00

Aspirin concentration

Day 7	
Epithelial-like	Intermediate
11.65	1.24
7.55	1.43
4.65	1.77
2.10	1.35
3.15	1.22
1.31	1.13
0.25	0.88
1.00	1.00

Immunostaining for CK and Vim



Scale bar is 20 μm

Cytokeratin (CK): a cytoplasmic marker for **epithelial** phenotypes
Vimentin (Vim): a cytoplasmic marker for **mesenchymal** phenotypes

Perillo B, Di Donato M, Pezone A, et al. Experimental & Molecular Medicine, 2020: 1-12.

Discussion

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- Compared with the treatment with doxorubicin alone, the **intracellular oxidative activity** in the sample under combinatorial **DA** treatment was **reduced**, as demonstrated by the intensity of Calcein AM. We demonstrated that the treatment outcomes were mediated by the reduction of COX-2, which was associated with inflammation triggered by **ROS**.

- Overall, the **preclinical model** could be used as a proof of concept to demonstrate the efficacy of anti-inflammatory combinatorial therapies by influencing oxidative stress. Similar research could provide a basis for more **DNA-related cancer treatment** research in the future.

Acknowledgements

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Microsystems for Personalised Medicine (MPM) Lab

Thanks to our lab members:
KHOO Bee Luan (Dr)
ZHANG Jing
DENG Yanlin
LIAO Junchen
FU Yatian
LI Wei
ZOU Shangjie
CHEN Chun kwan
WANG Shan
GUO Wang

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Thank you!