KIYATEC®

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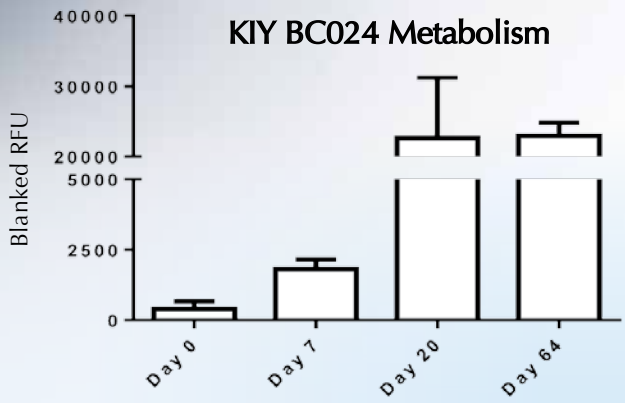
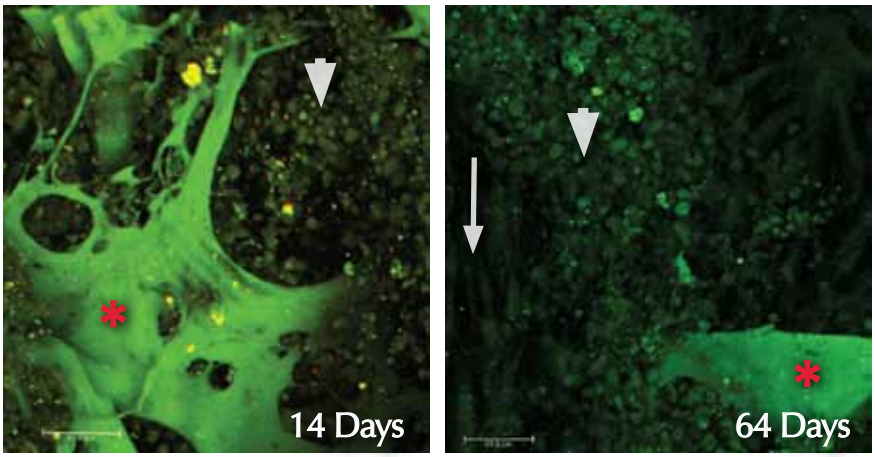
KIYATEC® Demonstrates Robust 64 Day 3D Primary Breast Tumor Co-Culture

In a 3D microenvironment, KIYATEC has cultured patient-derived triple negative breast cancer (TNBC) tissue for 64 days, demonstrating:

- In Vitro maintenance and robust viability of clinically-sourced, primary tumor cells for extended periods of time
- 3D microtumor morphology closely resembles primary tumor morphology via self-assembly of multiple different cell types
- Long-term effects of targeted drugs (i.e. epigenetic modulators) may be assessed in vitro
- 3D tumor microenvironment maintains key molecular subtypes
- Co-cultured fibroblasts did not overgrow primary tumor cells
- Functional analyses (i.e. phenotypic drug response) are feasible for many weeks
- Long-term viability enables modeling of resistance mechanisms and combination dosing



Cellular metabolism increases over time in 3D tissues. 3D tissues were maintained in culture for 64 days. Cellular metabolism was measured with incubation in PrestoBlue, a resazurin reduction assay. Data represents at least 3 replicates with standard deviation.



Multiphoton images of 3D microtumors over time. Multiphoton images were taken of 3D microtumors after both 14 and 64 days in static culture. Red asterisk indicates silk scaffold, white arrows indicate fibroblasts, and white arrowheads indicate tumor cells.

The KIYATEC® Advantage

KIYATEC's expertise in 3D co-culture techniques, scaffold material selection and complex tissue microenvironment biology has allowed the company to maintain viable TNBC microtumors for 64 days. Cell viability was monitored at Day 0, Day 7, Day 20 and Day 64 using a resazurin reduction assay tuned for 3D cell culture. Multiphoton images at Day 14 and Day 64 confirm the presence of viable tumor cells without significant overgrowth of fibroblasts at Day 64.

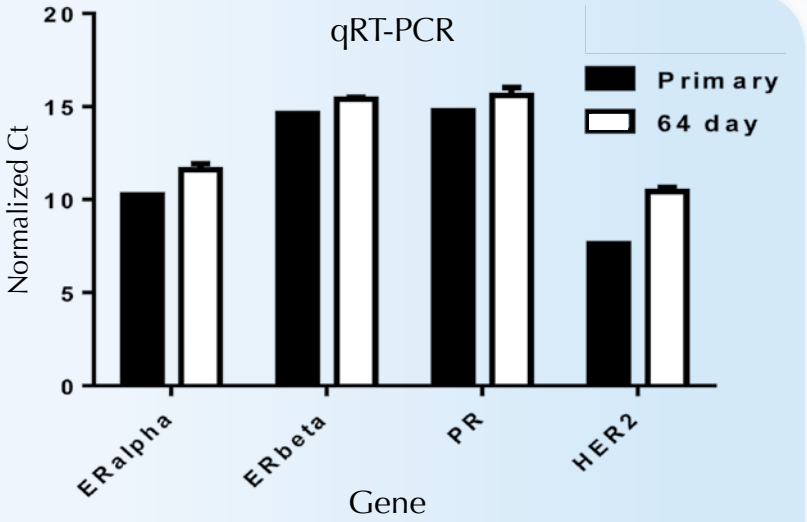
To confirm the molecular subtype remained after Day 64, primary tumor tissue and corresponding 64 day TNBC 3D microtumors were tested for mRNA expression by qRT-PCR. Importantly, ER, PR and Her2 expression levels were maintained after 64 days of culture. Histological analysis of the primary tumor compared favorably to the TNBC 3D microtumors at Days 14 and 64 and confirmed the presence of epithelial tumor cells in an *in vivo*-like microenvironment.

The KIYATEC® Approach Enabled:

- Long-term viability (>2 months) of Triple Negative Breast Cancer (TNBC) 3D microtumors
- 64 day TNBC 3D microtumors maintained mRNA expression similar to primary tumor sample
- Histological evaluation confirms presence of viable epithelial tumor cells after 64 days of culture

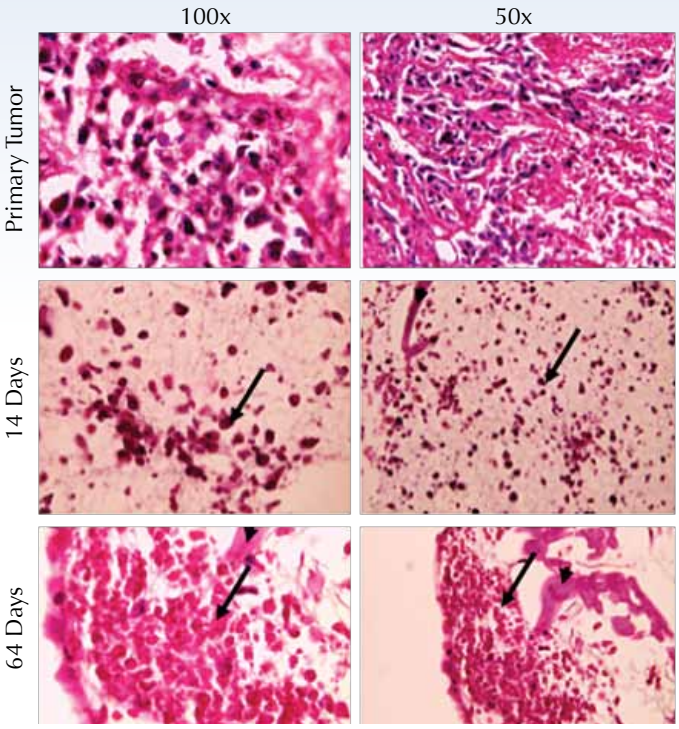
Average Ct

	64 day	Primary
ER alpha	29.90	28.69
ER beta	33.68	33.07
PR	33.89	33.22
HER2	28.73	26.06



Gene expression in 64 day 3D microtumor compared to primary tumor. RNA was isolated from both the primary tumor and the 3D microtumor following 64 days in culture. The expression of mRNA for ER, PR, and Her2 were analyzed to confirm the molecular subtype of triple negative in both the primary tissue and the 3D microtumor.

3D Microtumor Histology



Tissue morphology of 3D microtumors and primary tissues. A portion of the primary tumor and individual 3D mcirotumors were fixed in formalin, embedded in paraffin and stained with H&E to visualize the morphology. H&E confirmed the presence of epithelial tumor cells within the 3D microtumors. Arrows indicate the tumor cells and arrowheads indicate the silk scaffolds.

KIYATEC's 3D co-culture systems and in vitro phenotypic Drug Response Profiling (DRP) services enable our clients to select and advance only their most promising and clinically relevant drug candidates. We seek to address your most challenging drug development issues. Whether preclinical candidate selection, poor in vitro to in vivo correlation, drug resistance, combination dosing or overall poor clinical performance, KIYATEC provides a cost effective and efficient solution to minimize risk and maximize clinical performance across your drug development pipeline.

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