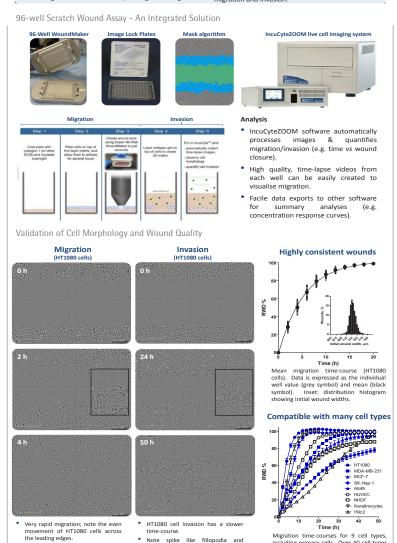


Differential Biology of Tumor Cell Migration and Invasion Through Bio-Matrices Measured with 96-Well Live-Cell Kinetic Imaging Assays

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Summary and Impact

- Cell migration and invasion is a pivotal event in a range of physiological and pathological processes including inflammation, wound healing & tumour development
- We have evolved the scratch wound method into an image-based, facile, robust, fully kinetic 96-well model of both cell migration and invasion.
- The approach is amenable to many cell types and screening of small molecules, biologics and gene-
- interference reagents (e.g. siRNA, miRNA).
- Kinetic analysis reveals temporal differences in the profile of different pharmacological agents.
- Differential pharmacology was seen when a bio matrix was included in the model. Notab matrix-dependent effects were also observed. Notably, bio
- This model displays morphological, temporal and pharmacological hallmarks of in vitro tumour cell



Migration and Invasion Time - Courses

Cell morphology remains consistent within and outside the wound.

96-well plate view HT1080 (invasive)

Note spike like fillopodia invasive tracks through v

neighbouring cells follow

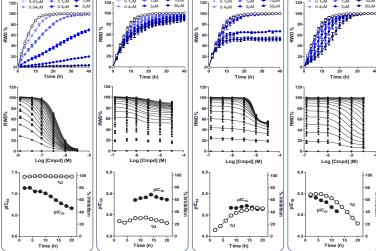
Migration vs invasion (BD-Matrigel™) 20 30 Time (b)

Migration time-courses for 9 cell types, including primary cells. Over 40 cell types

- Only invasive cell types, such as HT1080 and MDA-MB-231 cells
- enter the wounded area when ECM (Matrigel) is present in the wound. The time-course of invasion is considerably slower than migration and is gel density dependent
- Non-invasive cell types, such as MCF-7 cells, fail to invade the wounded area when ECM is present.

Cytochalsin D KU0063794 CCT018159 Wortmannin (Hsp90 inhil (PI3 kinase inhibitor) (actin polymerisation inh.) - 0.1μM -- 0.3μM -+ 1μM + + 3υM + 30 40

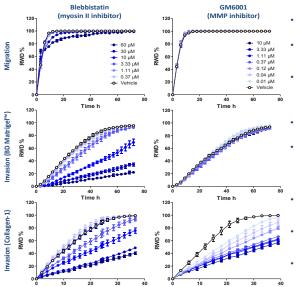
Cell Signaling Inhibitors Yield Different Temporal Profiles



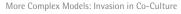
- HT1080 mean timecourse data expressed as %RWD vs time. Compounds added to cells immediately after wound creation (t=0). Data from four 96-well plates. Note immediate attenuation of migration by wortmannin, consistent with a role of PI3K in defining cell polarity and the leading edge. Note attenuation of later phases of migration by CCT018159 (Hsp90 inhibitor).

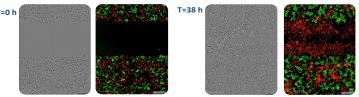
- Potency (pIC₅₀) and efficacy (% max inhibition) values were obtained at different time points from curve fits to the concentration-response data. Note decreasing potency and efficacy of wortmannin with time Note increasing efficacy of CCT018159 with time.

Differential Biology of Cell Migration and Invasion



- HT1080 cells migrate very rapidly when no ECM is present in the wound.
- Blebbistatin vields only small effects on migration and only at the highest concentration
- GM6001 (up to 10 μ M) does not effect the migration of HT1080.
 - The time-course of invasion into Matrigel is markedly slower than migration.
- Blebbistatin. but GM6001, inhibits HT1080 invasion through Matrigel in a concentration-dependent
 - The time-course of invasion into collagen-1 is more rapid that observed Matrigel.
- Blebbistatin inhibits HT1080 invasion through collagen-1 with a similar potency to that observed for Matrigel.
- GM6001 inhibits HT1080 invasion through collagen-in a concentration dependent manner.





MCF-7 (Green) + HT1080 (Red) non-invasive invasive

- The non-invasive MCF-7 cells fail to penetrate Matrigel, whereas the highly invasive HT1080 cells fully invade.

