The informational content of single-cell time-lapse imaging to study the response of human cells to therapeutic anti-cancer treatments

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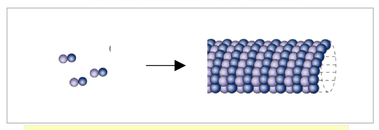
INTRODUCTION

Mitotic division is a target in anti-cancer therapy

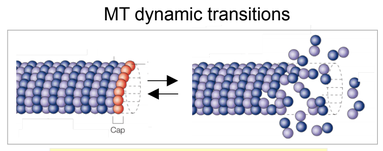
Anti-mitotic compounds are considered effective agents to eliminate highly proliferating tumor cells, by arresting cells in mitosis and inducing cell death

1. Anti-microtubule (MT) drugs that target either the assembly, or the dynamics of the spindle MTs are used to effectively arrest cancer cell proliferation.

MT formation from tubulin



MT dynamic transitions



MT-DEPOLYMERIZING DRUGS
Prevent **assembly** of mitotic MTs

vincristine, vinblastine
nocodazole, colchicine, combretastatin

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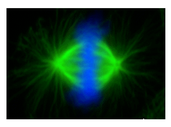

MT-STABILIZING DRUGS
Block MT **dynamic** functions

taxol (paclitaxel),
docetaxel

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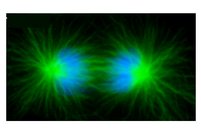
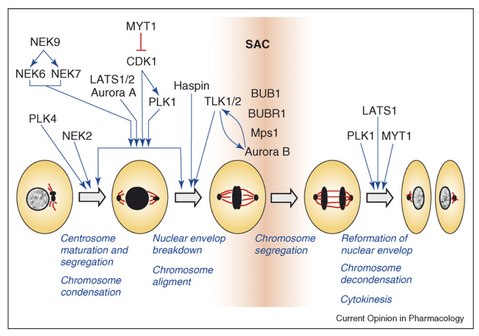


2. In addition to validated MT-targeting drugs, novel compounds, directed against **mitotic kinases**, are being developed and evaluated in clinical trials.

Kinases involved in mitotic division

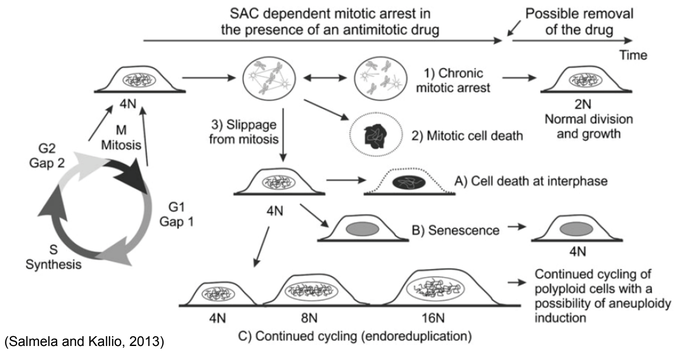


Suitability of mitotic kinases as therapeutic targets :

- specifically expressed in dividing cells (no toxic effects on interphases)
- often deregulated in tumors (advantage for diagnosis and choice of treatment)
- Presence of targeting sites for small molecule inhibitors ("druggability")

Cell-to-cell variability in response to anti-mitotic drugs

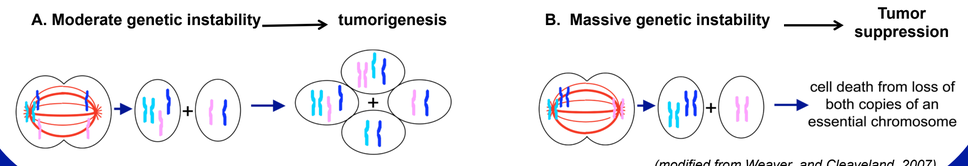
The introduction of single-cell analyses, e.g. in live cell imaging, has provided significant advance in the drug design field, evidencing a cell-to-cell variability that had gone unnoticed in whole cell population studies.



(Salmela and Kallio, 2013)

MITOTIC CATASTROPHE, a poorly understood death pathway, is the main form of cell death response to antimitotic drugs, to eliminate genetically unstable cells.

Aneuploidy can drive or inhibit tumorigenesis: since responses to anti-mitotic drugs are heterogeneous, getting a better understanding is important to predict whether these compounds will have clinical efficacy



(modified from Weaver and Cleaveland, 2007)

AIM

Developing quantitative high-resolution imaging methods, including at the high-throughput level, to characterise the behaviour of single cells treated with potential anti-cancer molecules

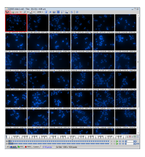
METHODOLOGY

INSTRUMENTAL SETTINGS

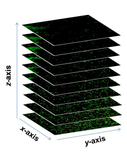
Nikon Eclipse Ti at the Nikon reference center at IBPM-CNR

- NIS-Elements HC (High Content Analysis)
- supports for simultaneous analysis of several conditions
- complete motorization
- multidimensional acquisition:

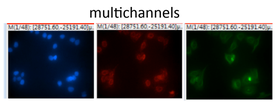
xy multipoints



Z-stacks

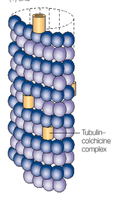


multichannels



ANTI-MITOTIC COMPOUNDS

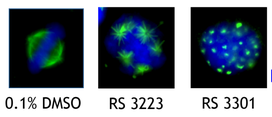
ARYLTHIOINDOLE TUBULIN POLYMERIZATION INHIBITORS (ATIs)



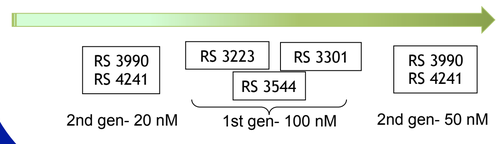
Colchicine: very effective binding to tubulin, but toxic

ATI STRUCTURAL DESIGN

1. based on the structure of **colchicine**-binding pocket on MTs
2. capable of **displacing colchicine** from MTs
3. small molecule class (MW < 500)
4. Addition of stabilizing lateral chains that can confer resistance to esterase enzymes



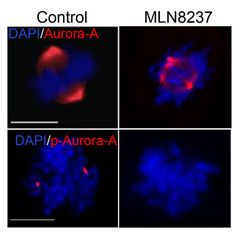
Inhibition of MT polymerization (24 h of treatment)



AURORA-A KINASE INHIBITORS

Aurora-A small chemical inhibitors directed against the ATP-binding site in the catalytic domain (ATP-competitors) are currently in clinical trials

Inhibitor	Tumor types	Current status
ENMD-2076	myeloma, breast cancer, leukemia, colorectal cancer, ovarian cancer	phase I/II
MLN-8237	lymphoma, leukemias, myeloma, breast cancer, prostate cancer	phase I/III
MK-5108	breast cancer, cervix cancer, colorectal cancer, ovarian cancer, pancreas neoplas	phase I
XL-228	lung, leukemia	phase I
KW-2449	leukemia	phase I

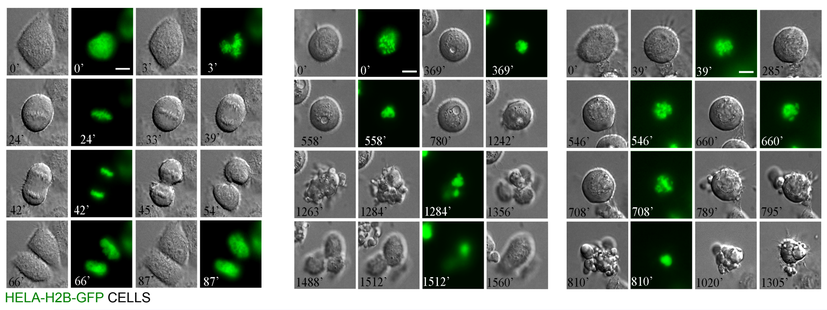


RESULTS

1. Recording individual cell fates by time lapse microscopy

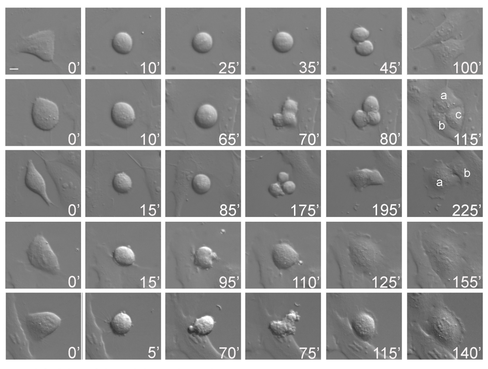
EXAMPLES OF RECORDED PHENOTYPES AFTER ATI TREATMENT

Control: normal cell division Example 1: **unbalanced cell division** Example 2: **mitotic cell death**



HELA-H2B-GFP CELLS

EXAMPLES OF RECORDED PHENOTYPES AFTER MLN8237 TREATMENT



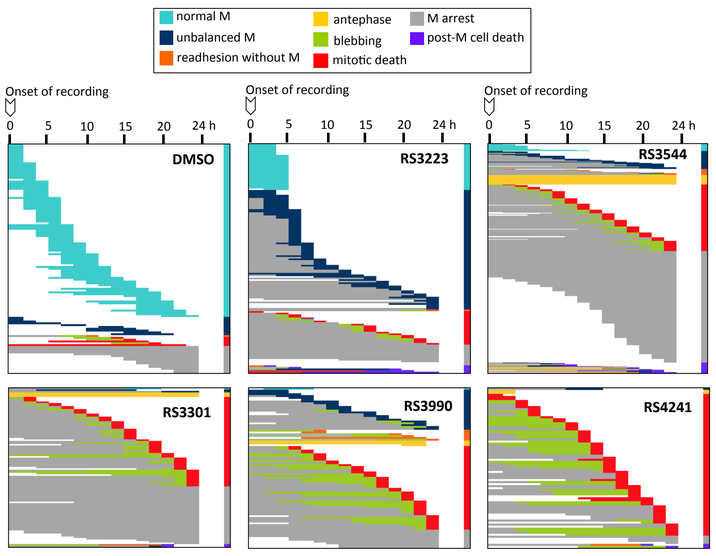
Control: normal cell division

Example 1: **unbalanced cell division**

Example 2: **no division**

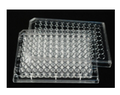
U2OS OSTEOSARCOMA CELLS

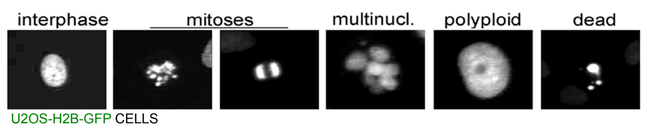
2. Single cell analysis after ATI treatment: true mitotic cell death is not necessarily dependent on the length of the mitotic arrest



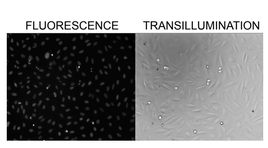
The timing of cell death in MT-damaged cells was recorded by time-lapse microscopy: the induction of mitotic catastrophe does not appear to be strictly dependent on the mitotic arrest duration but rather on the extent of MT damage

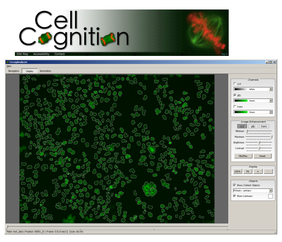
3. A high-throughput video-recording approach and automated analysis to follow the fate of MLN8237-treated cells over time

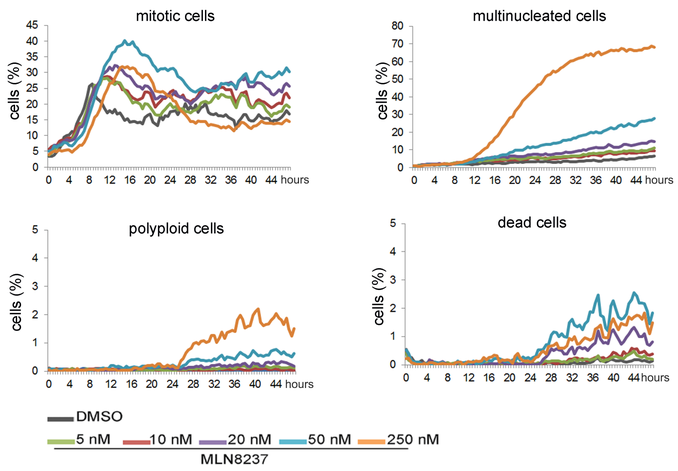




U2OS-H2B-GFP CELLS





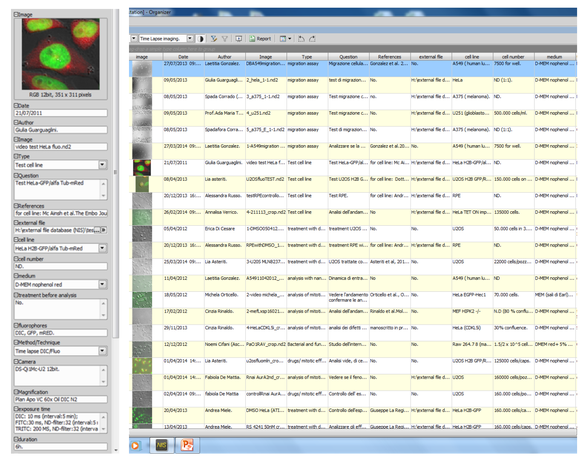


Proliferation of MLN8237-treated cultures is slowed down in a dose-dependent manner, without significant induction of cell death neither from mitosis or the following interphase within 44 hours from the treatment, and paralleled by an accumulation of multinucleated cells.

(Asteriti et al., 2014)

EURO-BIOIMAGING EMBL Advanced Light Microscopy Facility

4. Our time-lapse imaging database



General information: Date, Author, Image, Type, Question, Exyernal file, Comments, References.
Experimental conditions: Cell line, Cell number, Supports, Treatment before analysis.
Enviromental conditions: Medium, Anti-evaporation oil.
Microscope settings: Duration, Method/Technique, Magnification, Fluorophores, Exposure time, XY points, Z slices

Experimental categories

- Test cell line
- Treatment with drugs
- Mitotic effects
- Bacterial and or nanoparticles uptake
- Migration assay (single cell or wound healing)
- Cell differentiation

We created a "time-lapse imaging" database, which includes optimized parameters for each experiment for reproducibly, comparison to standards, recording of both "manual" and automated annotations

CONCLUSIONS

Our studies highlight the power of imaging approaches in drug development and screening protocols:

- We depicted the complexity of the cellular response to anti-mitotic drugs of potential therapeutic value, evidencing stochastic effects that may lead to aneuploidy, a potentially pro-tumorigenic condition.
- We highlighted important cell fate differences depending on the drug concentration, which may influence the outcome of the treatment
- We characterized dynamic parameters of mitotic catastrophe, the main death pathway in response to aberrant mitotic division.

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CNR InterOmics ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO AIRC PRIN 2010 - 2011 Nikon