High throughput Drug Library screening for modulators of G_2/M checkpoint by flow cytometry

NFCF meeting 19.-20. May 2016

Kari-Anne Myrum Frikstad

Cancer and treatment

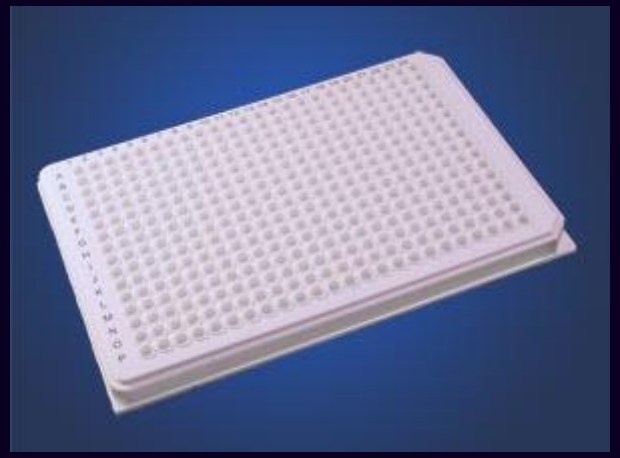
- Cancer treatment of today:
  - Surgery
  - Radiation
  - Chemotherapy

- Knowledge on molecular mechanisms → Better treatment

- Drug screening goals:
  - New uses for approved drugs
  - Pathways affected in a given cancer
  - Suitable targets for future drugs

HTS screening protocol – automation

Drugs printed in 384 well plates
5 µl/well for f.c.. 10 µM



(Done by The Biotechnology Centre of Oslo)

Sample handling:
seeding, fixation, staining, etc.

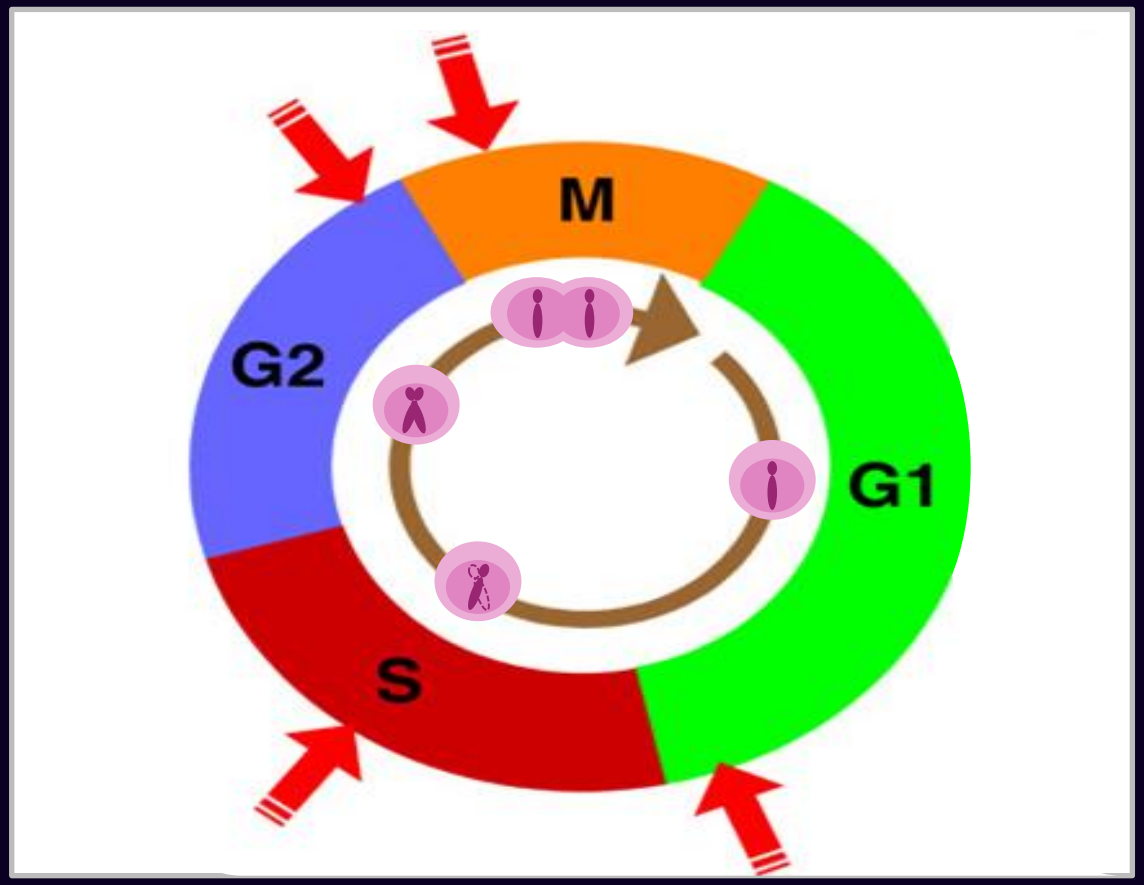
High throughput
analysis by flow cytometry

Drug Libraries

- Library of Pharmacologically Active Compounds – LOPAC
  - Sigma Aldrich
  - 1280 drug-like molecules
    in the fields of Cell Signaling & Neuroscience

- Cambridge Cancer Compound Library
  - SelleckChem
  - 384 anti-cancer compounds

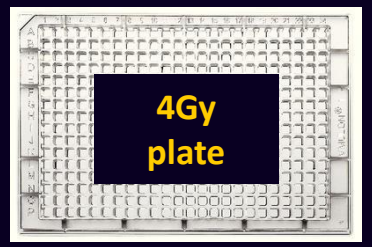
Cancer and the cell cycle



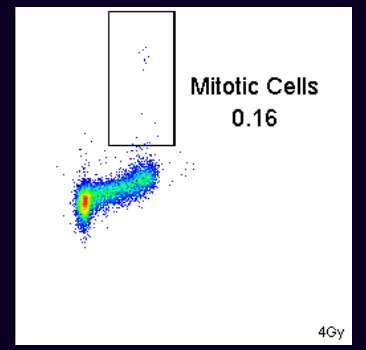
Eishi Noguchi, 2006 (modified)

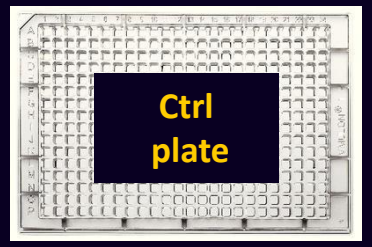
- Dividing cells go through the cell cycle and are closely monitored by cell cycle checkpoints
- Checkpoints detect DNA damage
- Cancer cells have acquired the ability to overcome the checkpoints

HTS screening protocol – experimental setup

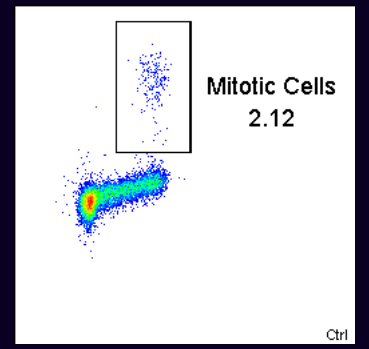


1 h incubation → **4Gy** 160 kV → 1 h incubation → MeOH fix → Stain → HTS Flow cytometry



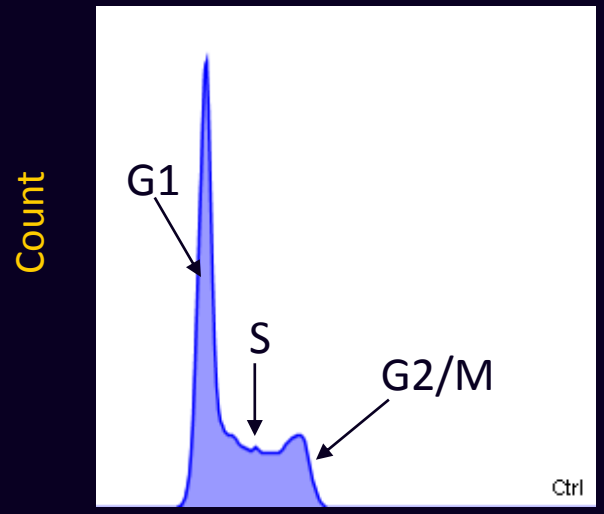


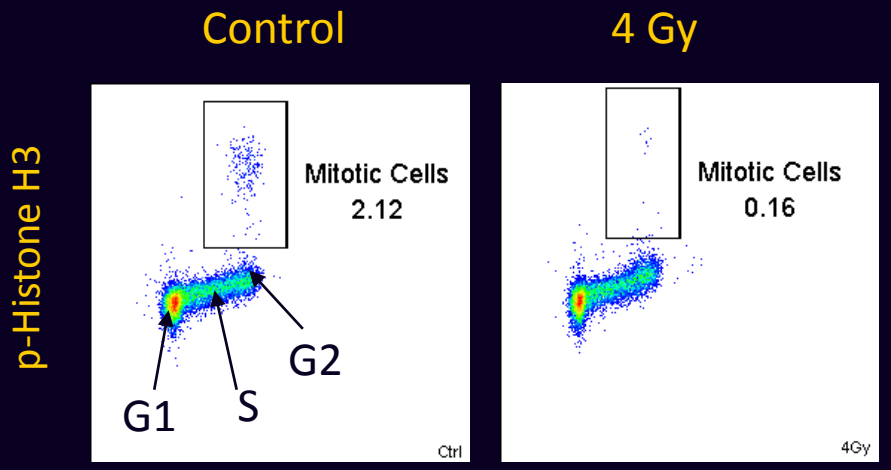
1 h incubation → **Mock** → 1 h incubation → MeOH fix → Stain → HTS Flow cytometry



End Point

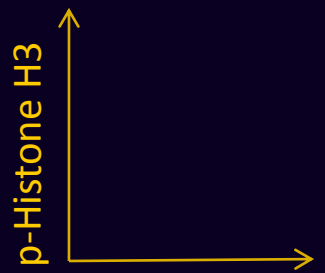
- Hoechst stain → Cell cycle distribution
- pH3 stain → Mitotic fraction
- Compare % mitotic cells in control and 4Gy

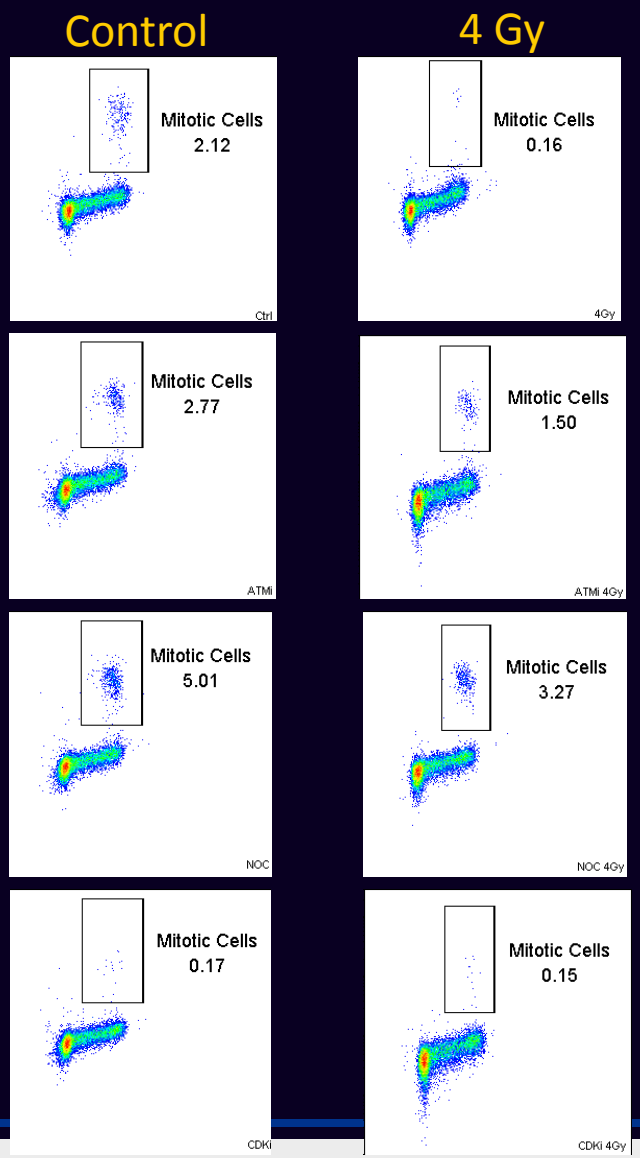




Analysis of results

➢ **Cambridge library**





Ctrl
(no drug)

KU-55933
(ATM inhibitor) → G2/M modulator

Nocodazole
(microtubule inhibitor) → Arrest in mitosis

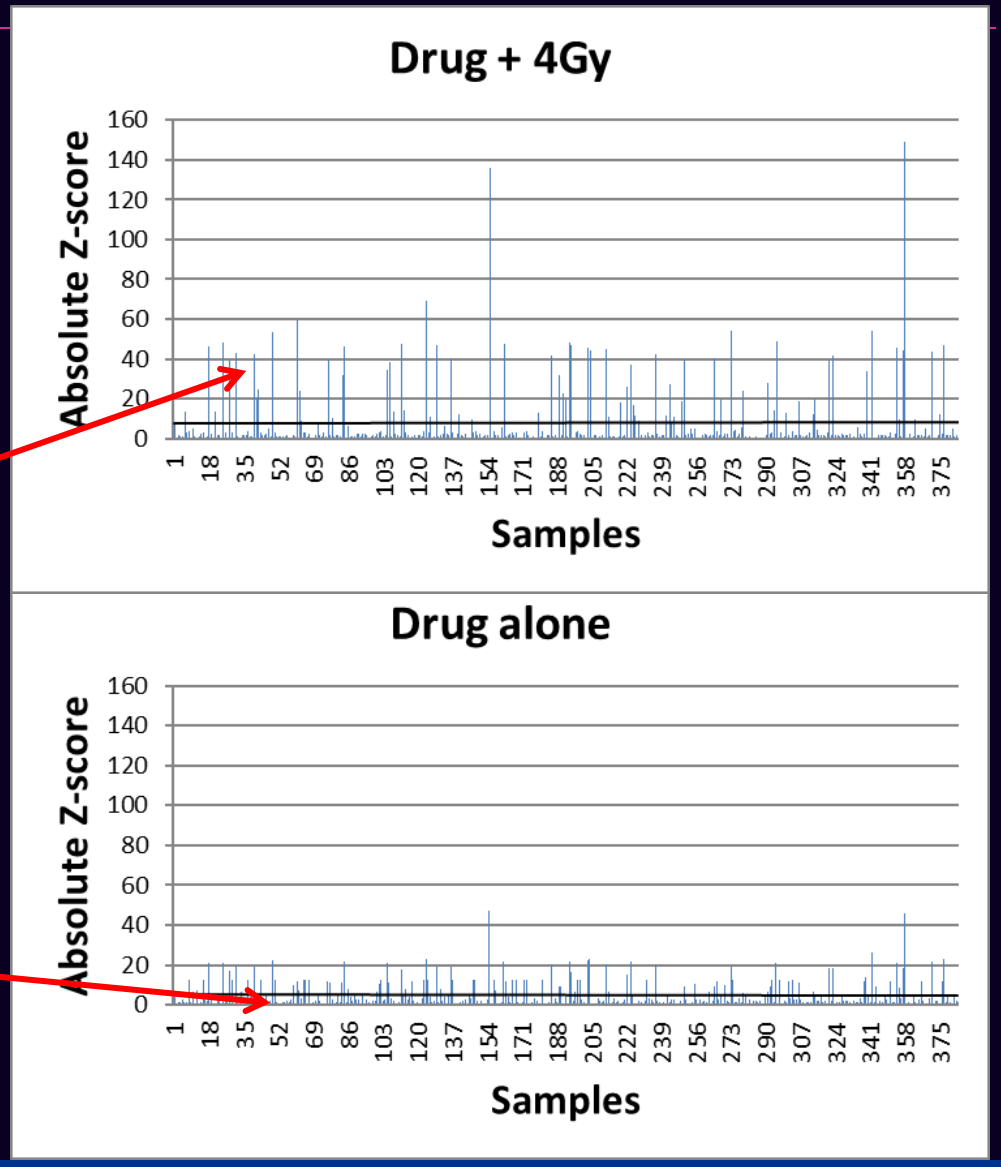
PHA793887
(CDK inhibitor) → Arrest in G2, not due to DNA damage

Analysis of results

- Z-score:

  Measure of how many standard deviations the change in mitotic fraction corresponds to when comparing samples with drugs +/- 4Gy

$$\text{Hit} = |Z_{4Gy}| > 4 \text{ and } |Z_{Ctrl}| < 4$$



Further investigation of hits

- HTS screen done in Reh and U698 cells
- Investigated the list of hits and selected some interesting hits to follow up
- Selected hits were validated by
  - Titration of drug concentration
  - Repeating experiment manually in tubes
  - Investigating further to find signaling pathways affected

Credits

Oslo University Hospital

Institute of Cancer Research

Department of Radiation Biology

- **Trond Stokke**
- **Petraz Juzenas**
- **Idun Dale Rein**
- Sebastian Patzke
- Monica Bostad

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University of Oslo

The Biotechnology Centre of Oslo

- Kjetil Tasken
- Anne Jorunn Stokka

Supplementary: Instruments

Robots:

- ELx405 Select (BioTek)
- PrecisionXS with Precision Power V2 software (BioTek)

Centrifuge:

- Allegra R21 Centrifuge (Beckman coulter)

Flow cytometer:

- LSR II (BD biosciences)
  - Red laser (633nm), Blue laser (488nm), Violet laser (405nm), UV laser (355nm)
- High Throughput Sampler (BD biosciences)