A new approach for animal free carcinogenicity testing using MN as biomarker (PMNvit)

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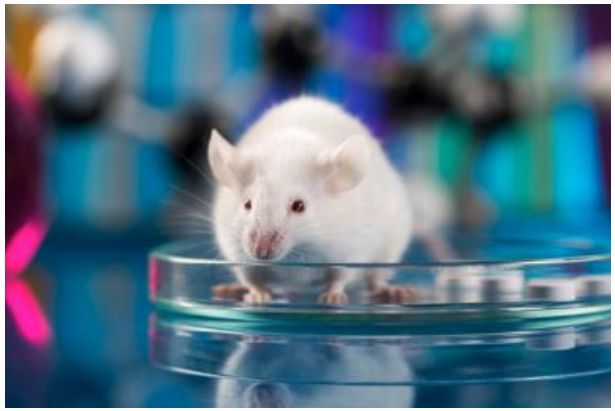
Overview

- The challenge: Predicting cancer risk
- State of the art (harmful animal testing)
- How to overcome the disadvantages of animal testing
- *In vitro* strategies derived from systems biological approaches (Learning *in vivo* but testing *in vitro*)



http://blog.humanesociety.org/wayne/2016/05/usda-shuts-major-animal-testing-enterprise.htmlPhoto by iStockphoto

Criticism of animal testing

- Replaceable high dose exposure experiments are still performed to recognize early warning biomarkers *in vivo*
- Mainly rodents are exposed for the majority of their life span
- Identification and classification of carcinogenic transformation/tumours is poorly standardized

https://gamesageddon.com/stock/media?id=81038656

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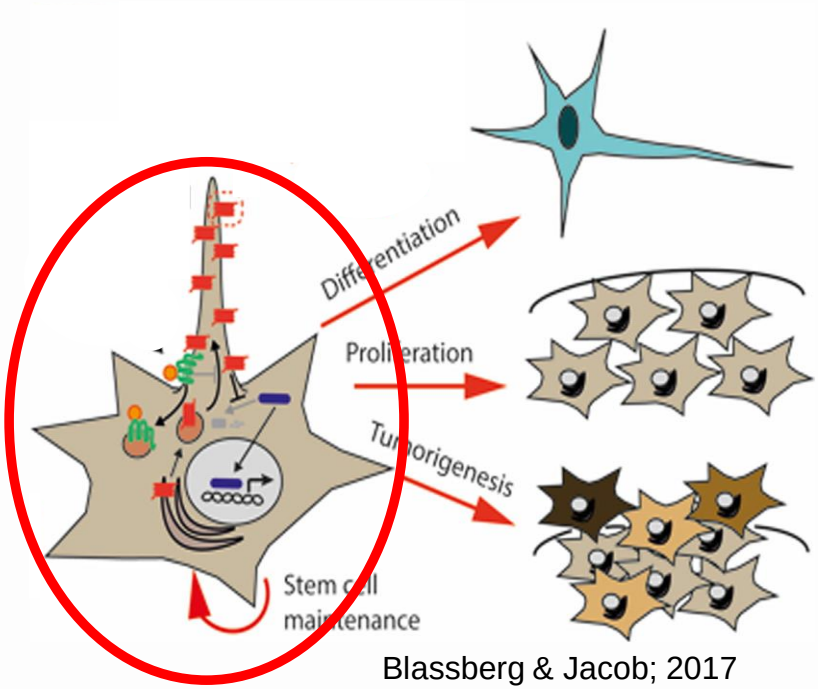
Regulation is based on the detection of tumours

- Does the test organism live long enough?
- Do dormant unnoticed early tumour stages exist?

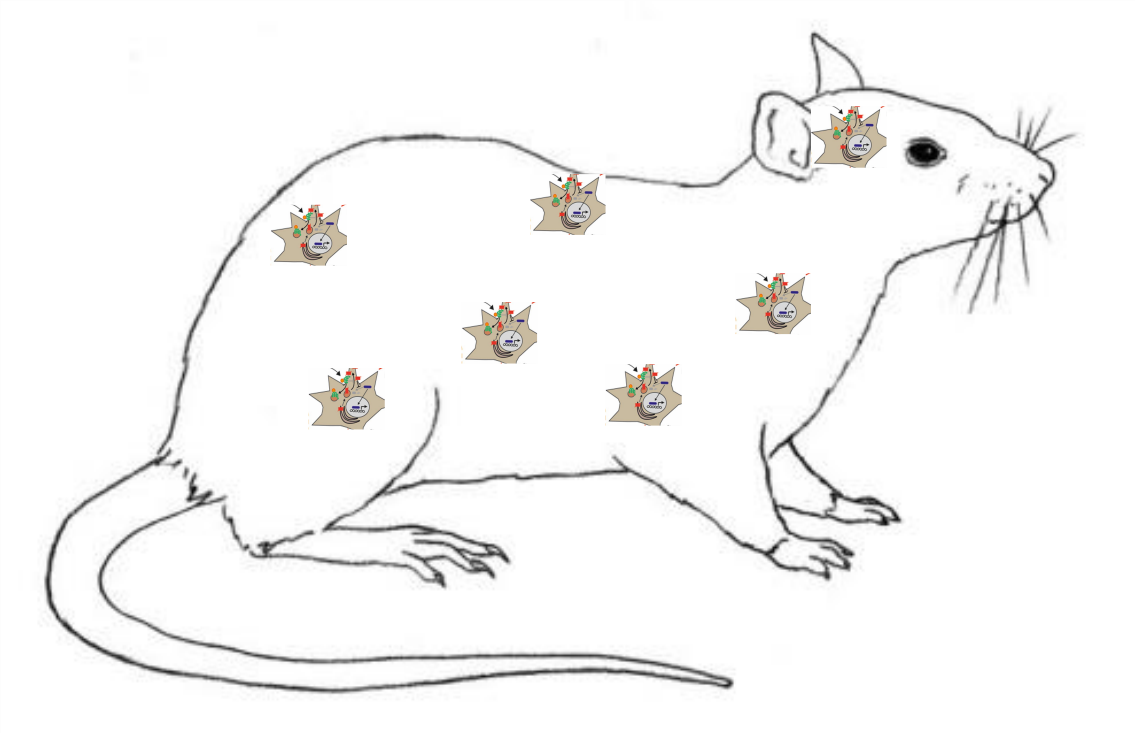
Existing limitations of *in vitro* toxicology

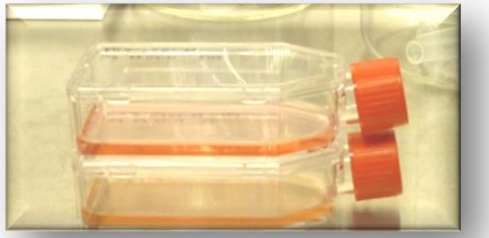


Blassberg & Jacob; 2017

- No appropriate cell lines available
- Most of the culturable rodent cell lines are immortalised through hybridisation with cancer cells or are extracted from tumours

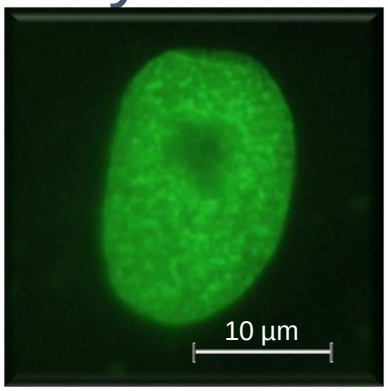
The rationale for animal testing

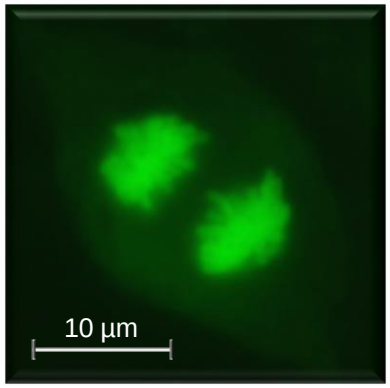


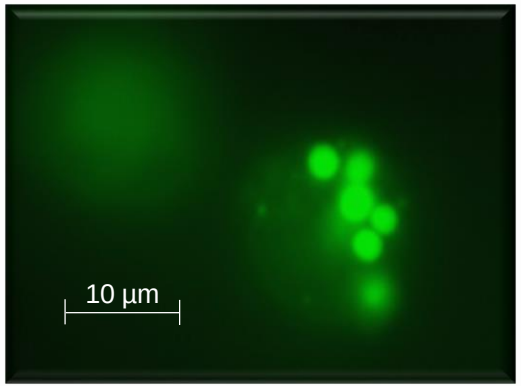


Criteria for healthy stem cells

- Normal control of cell cycle
- Responsive to cell signalling
- Susceptible for malignant transformation
- Able to differentiate
- Ability to die in course of cellular turnover

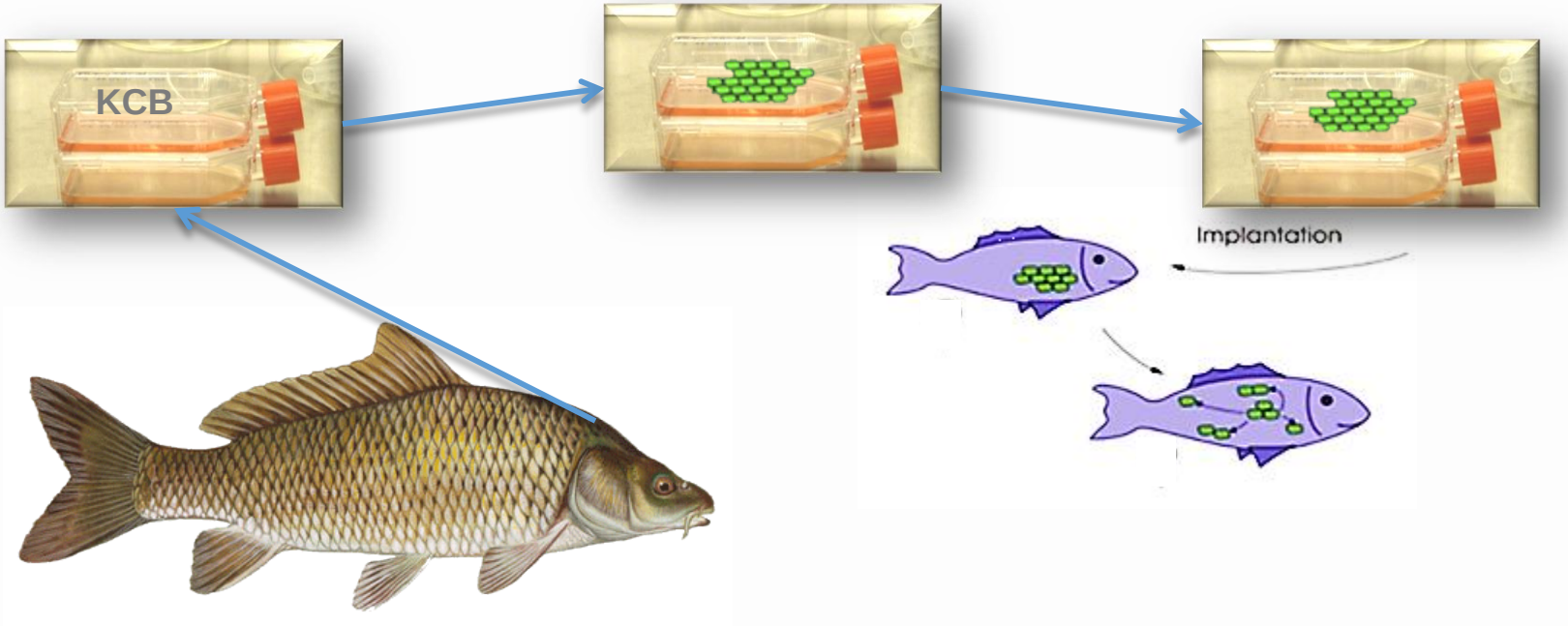




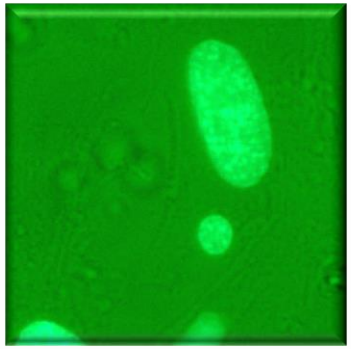


KCB-Cells

KCB-GFP = DSM ACC3285 Budapest treaty
26°C growth temperature / no CO_2



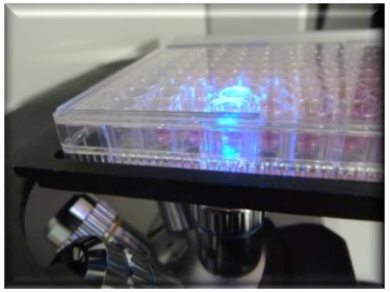
Technical opportunities with the KCB cell line



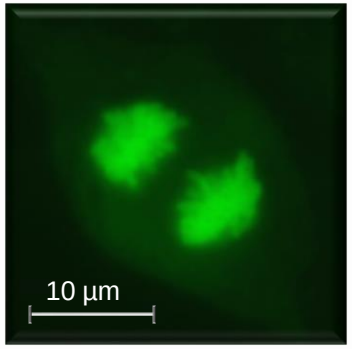
- First application

  get information on the health status;

  micronucleus formation and apoptosis



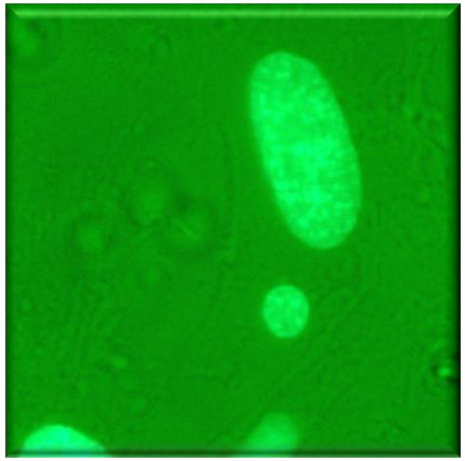
- Analysis of long-term inherited alteration of the cells e.g. pyknotisation

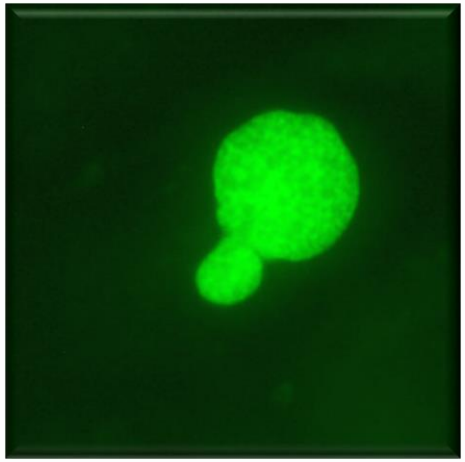


- Kinetic live imaging of cell cycle related events is possible

The cancer initiating events

1. Induction of micronuclei can be caused by chemically or physically induced strand breaks & chromosome loss as well as mechanical stress caused by cell migration
2. Chromothripsis ⟶ re-integration of micronuclei causing clustered gene translocation

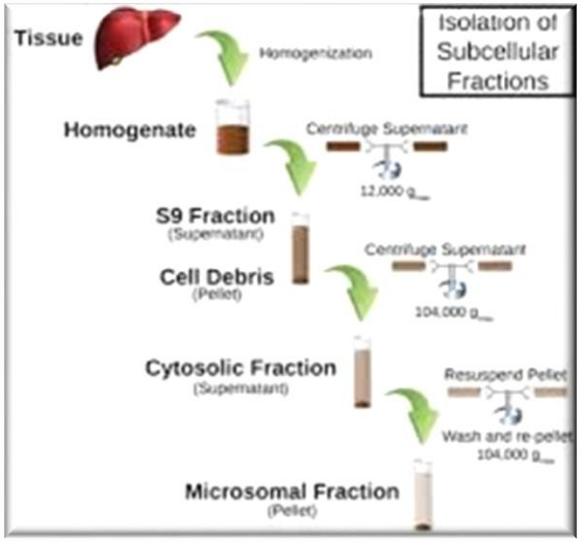




Overcome the S9 fraction *in vitro* deception

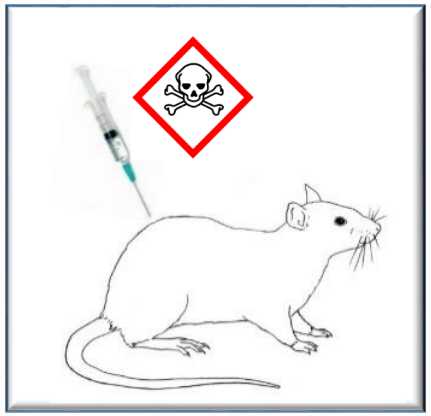
Poisoning of animals

for performing *in vitro* tests!



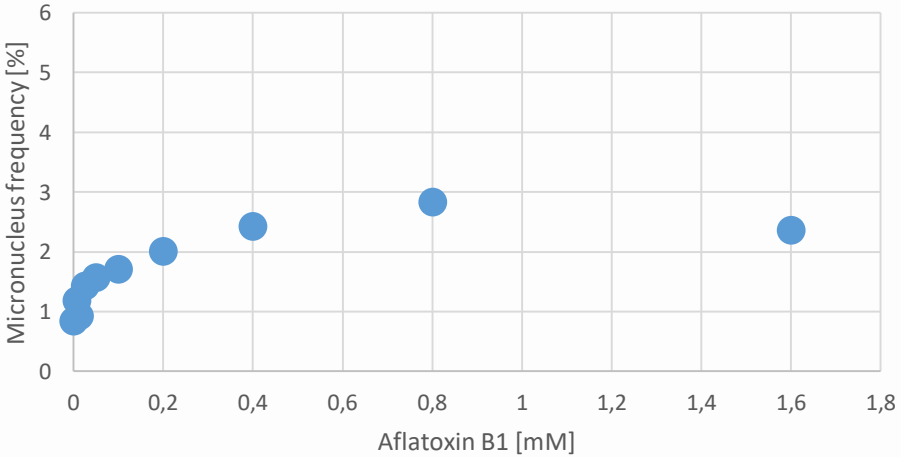
https://www.xenotech.com

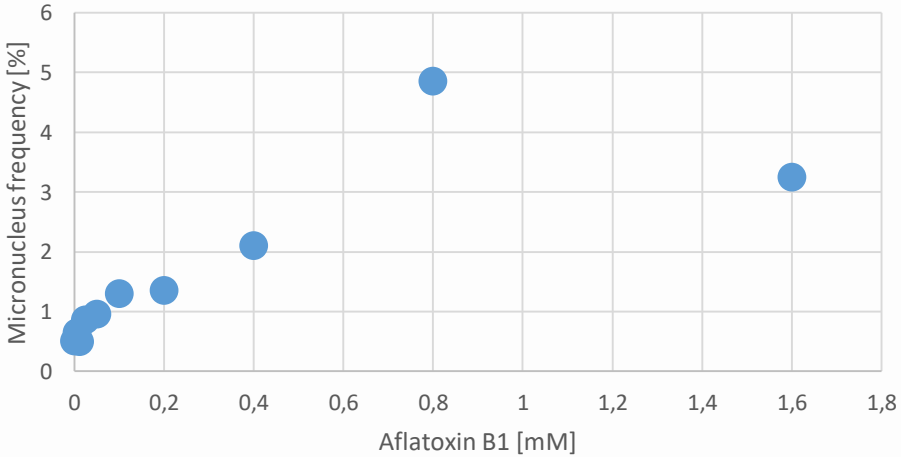
MN Results



Aflatoxin B1 + S9

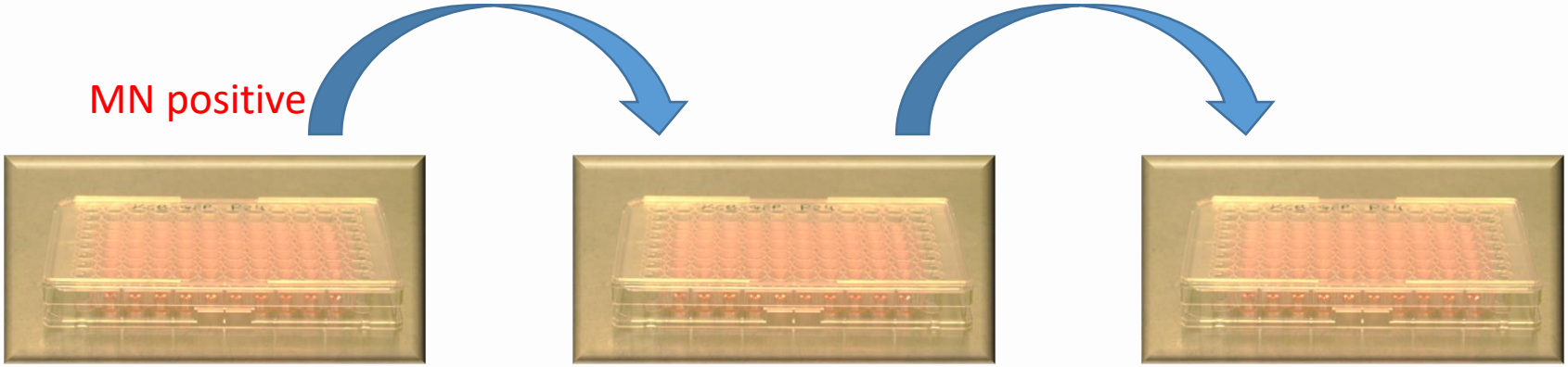
Aflatoxin B1 + EWOMIS S9



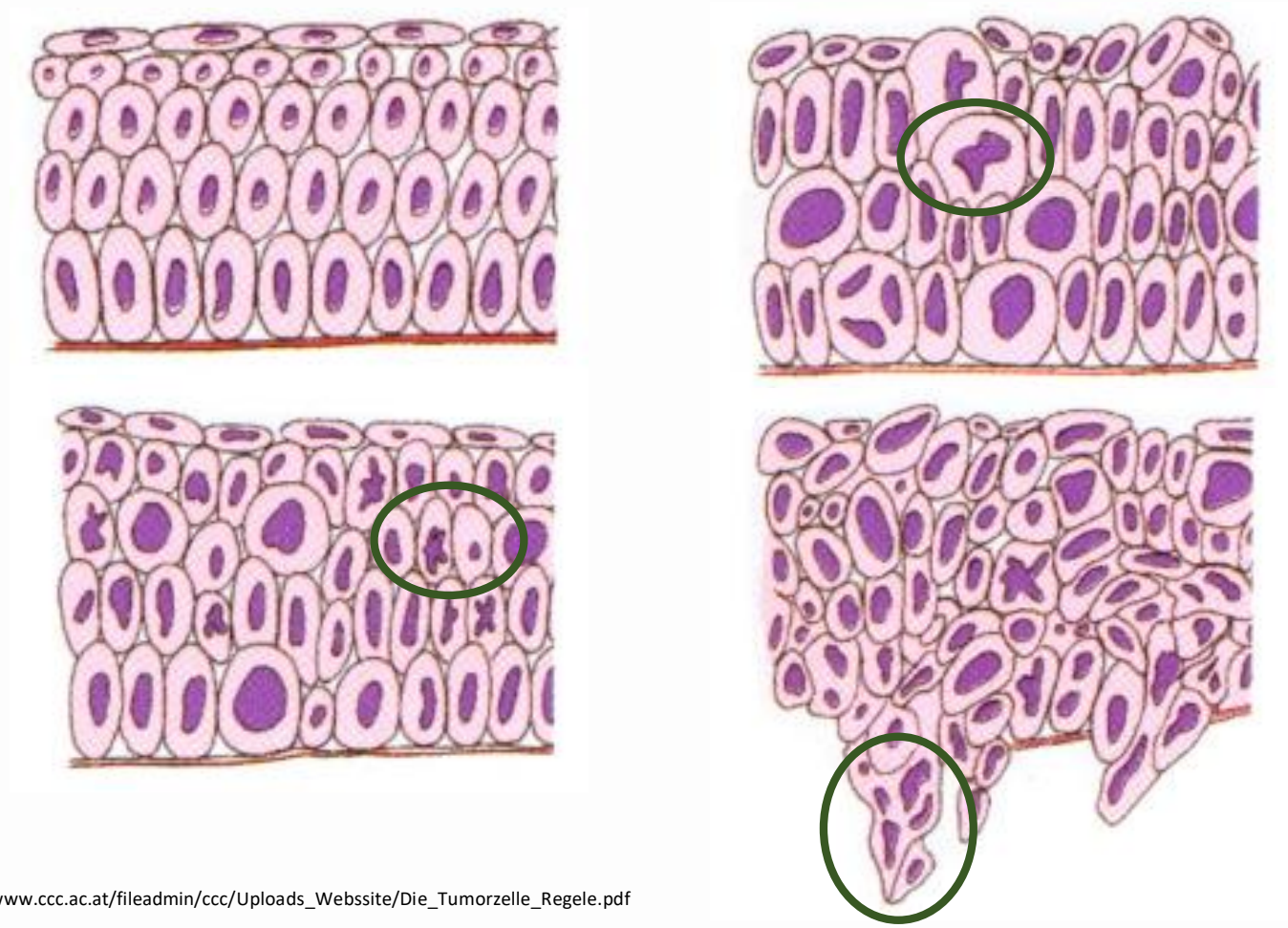


Downstream-Analysis

- Cells are exposed to a putative carcinogen for the duration of one cell cycle (24h) or permanently
- Cells were passaged and analysed once per week

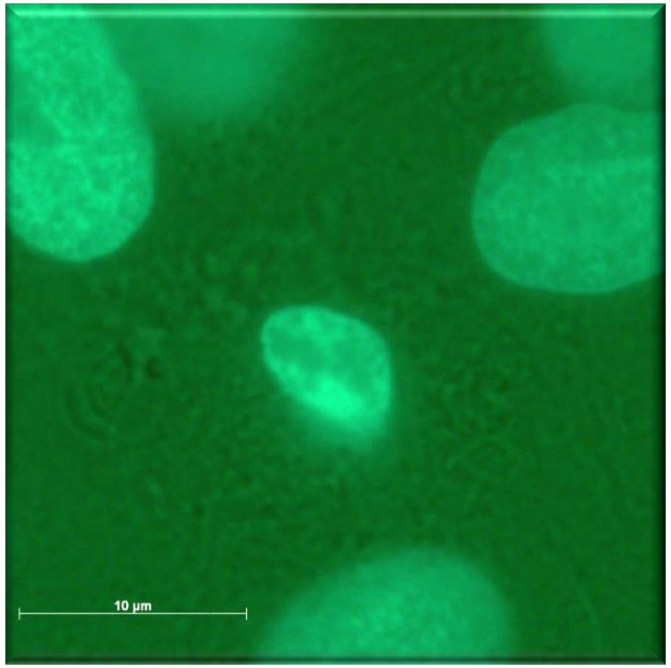


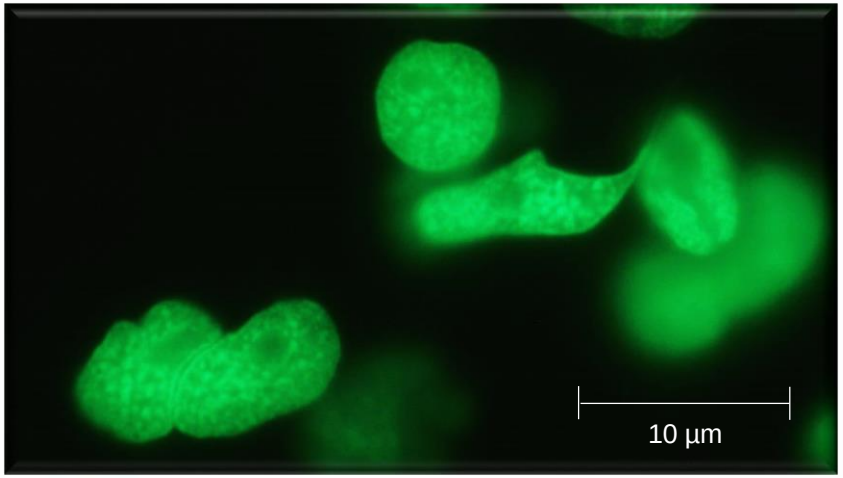
Malignant degeneration

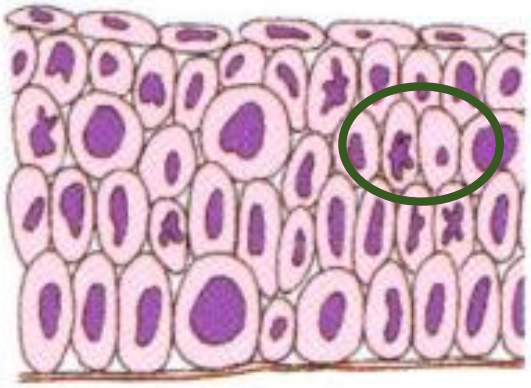


https://www.ccc.ac.at/fileadmin/ccc/Uploads_Webssite/Die_Tumorzelle_Regele.pdf

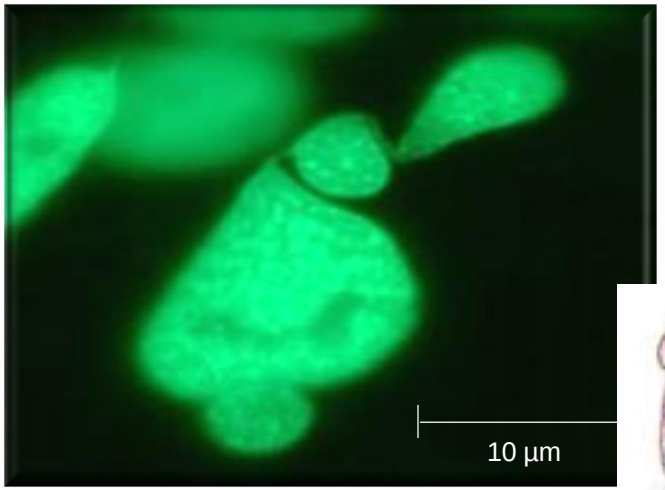
Pyknotic and deformed nuclei

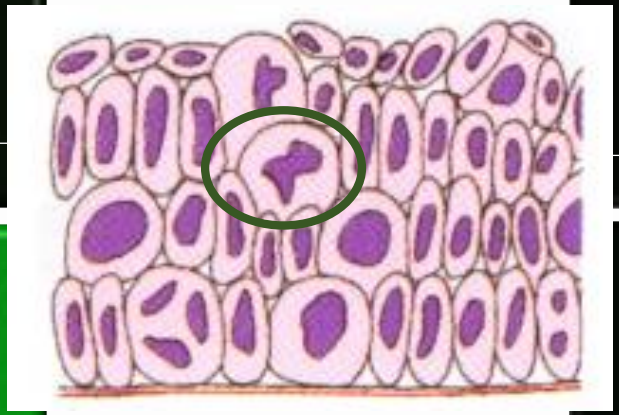


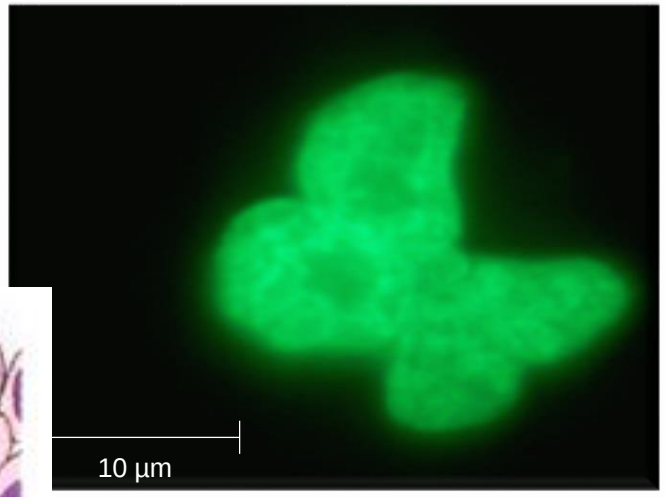


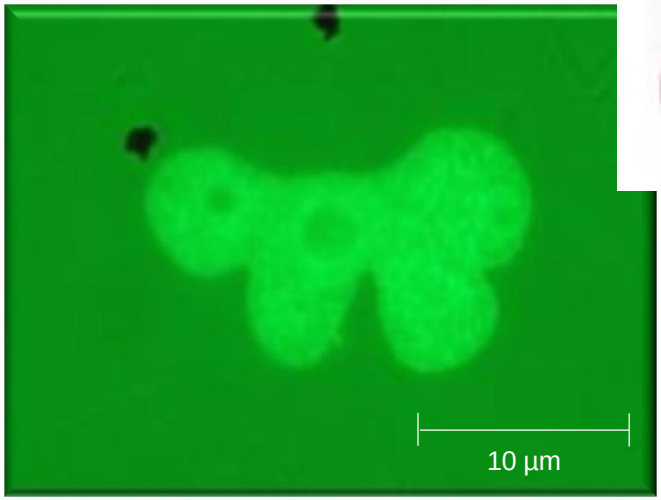


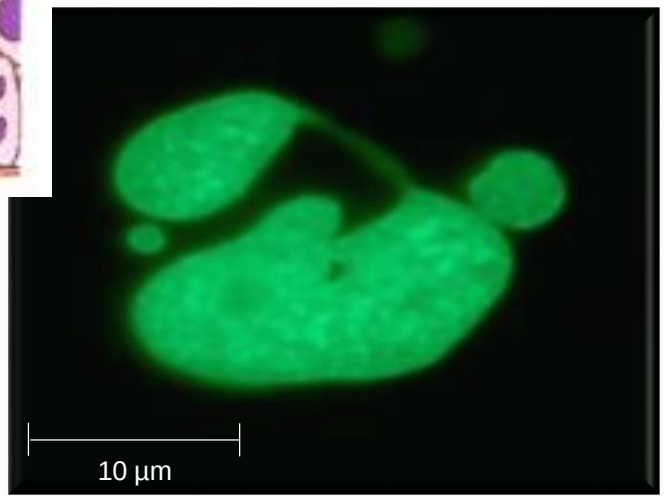
Multi-lobed nuclei



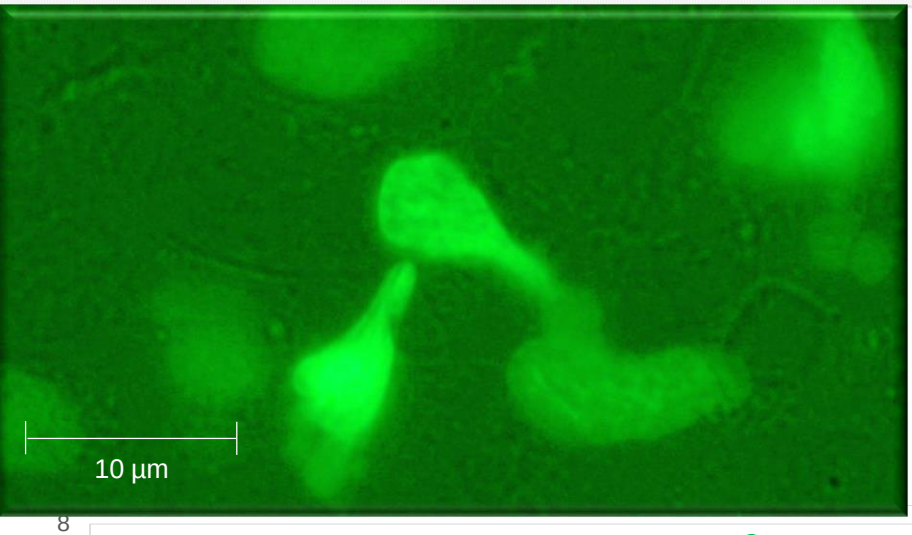


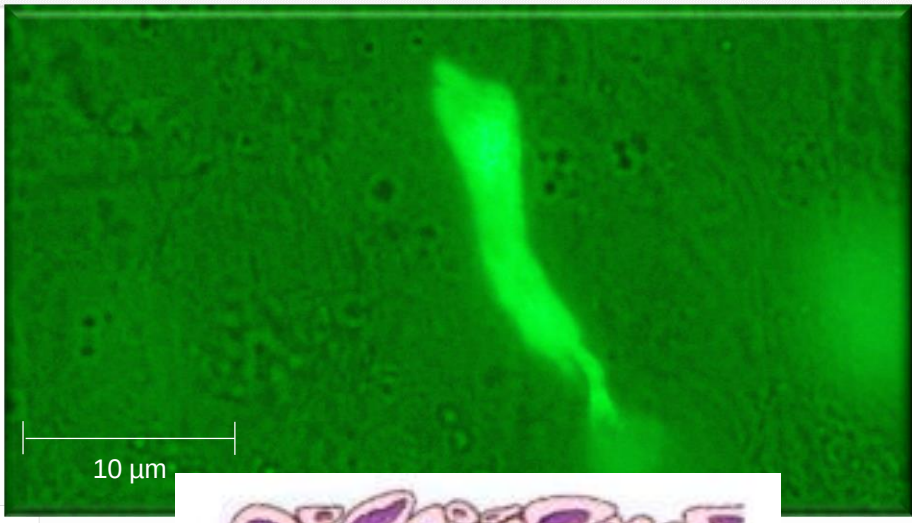


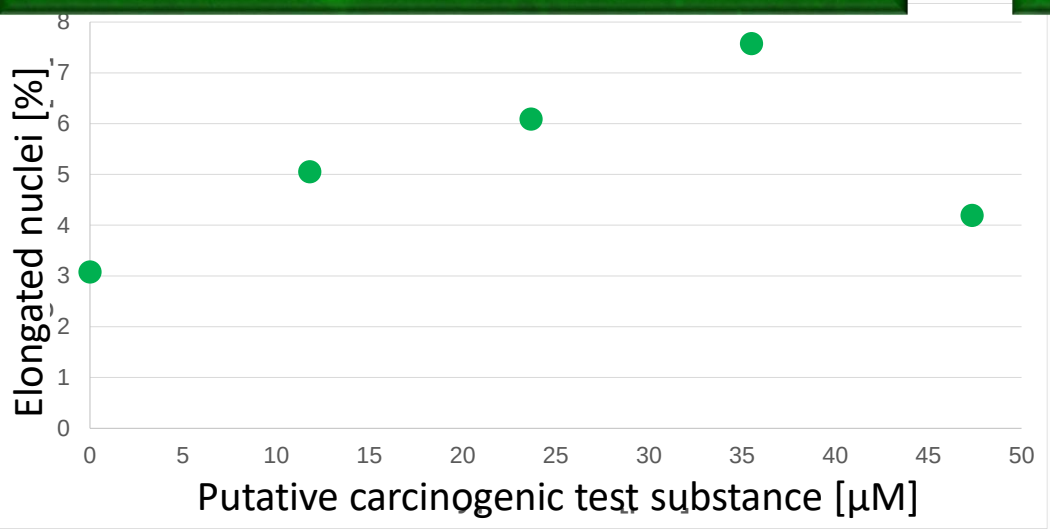


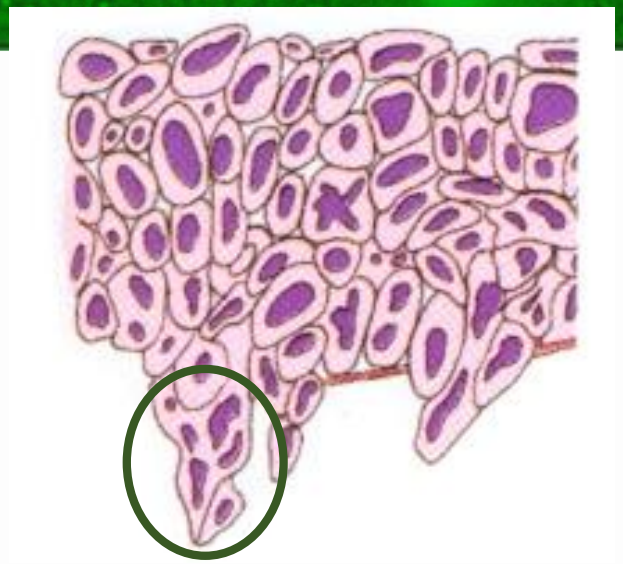


Elongated nuclei

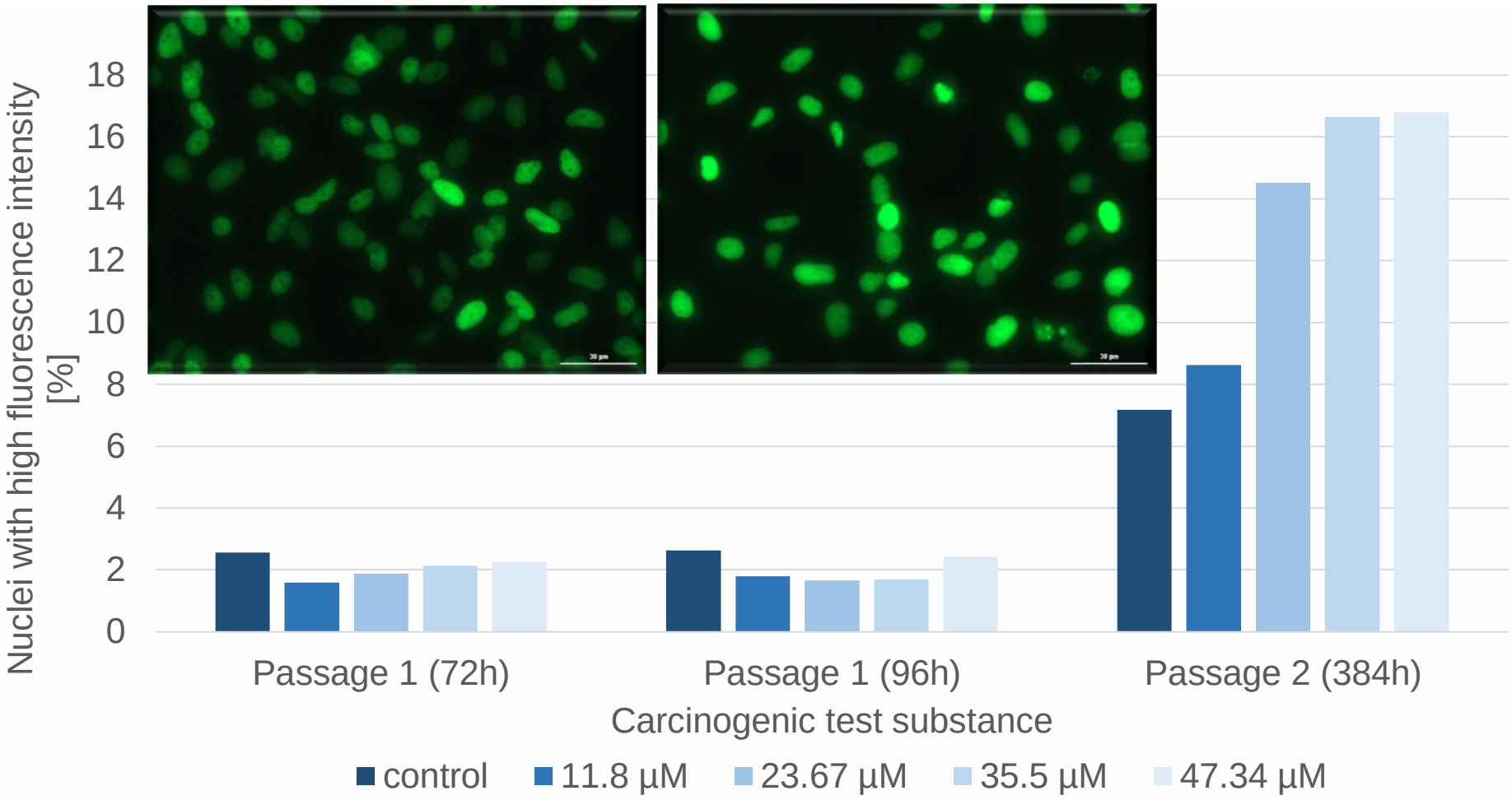




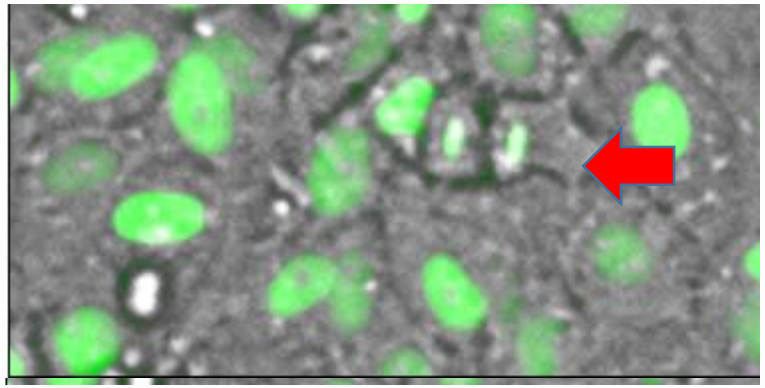


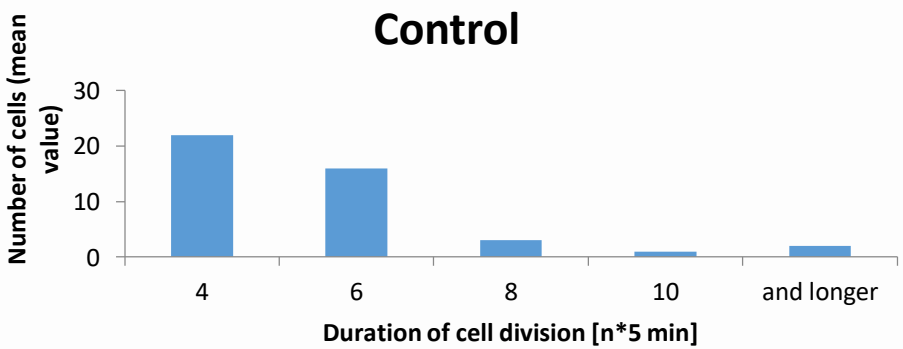


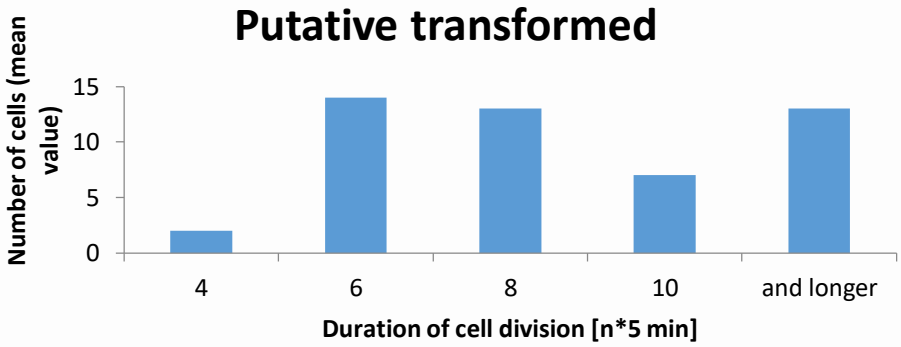
Increase of fluorescence intensity



Proliferation analysis

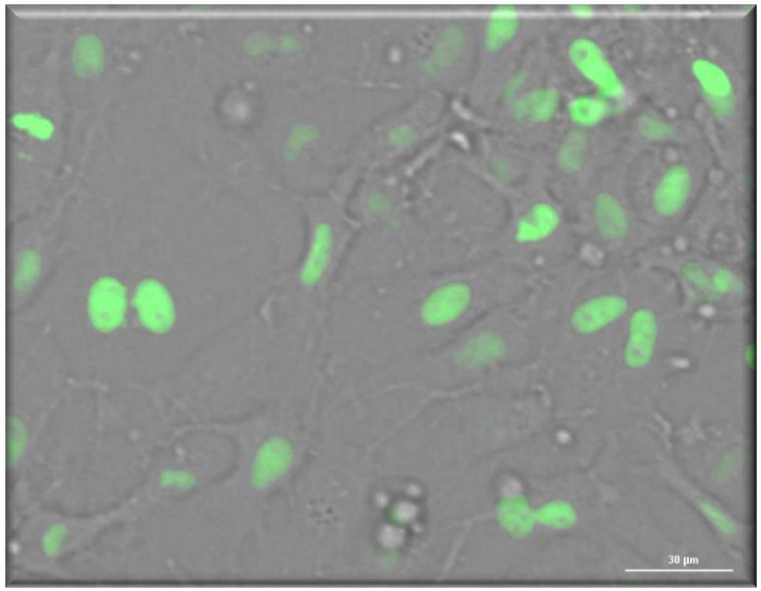






Future perspectives and research needs

Using healthy stem cells in sound *in vitro* studies is the smarter approach to understand malignant degeneration



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Thank you very much for your attention!