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

Bastian N. Hölzel

## 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.html> Photo 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



B. N. Hölzel



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

#81038656

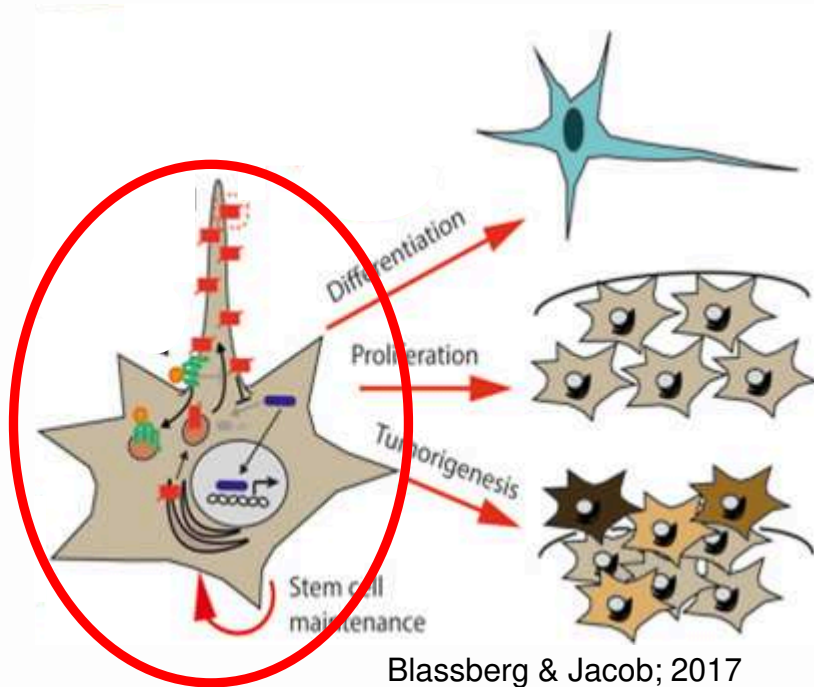
## Testing of genotoxic/carcinogenic substances

Regulation is based on the detection of tumours



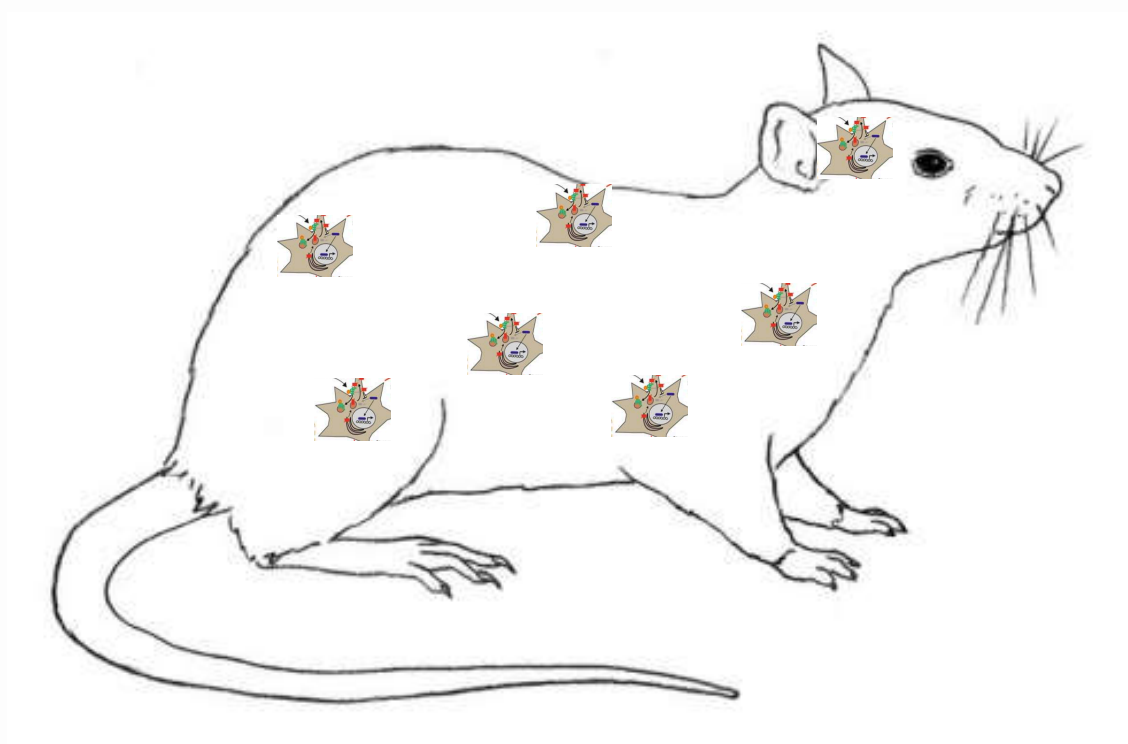
- Does the test organism live long enough?
- Do dormant unnoticed early tumour stages exist?

## Existing limitations of *in vitro* toxicology



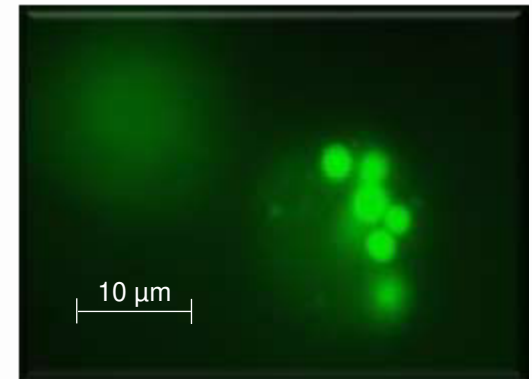
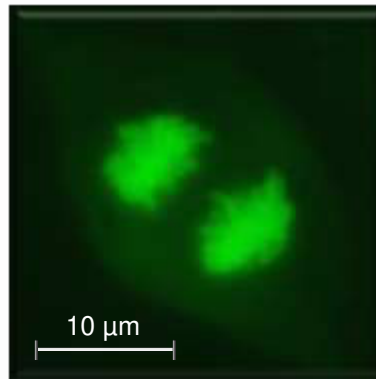
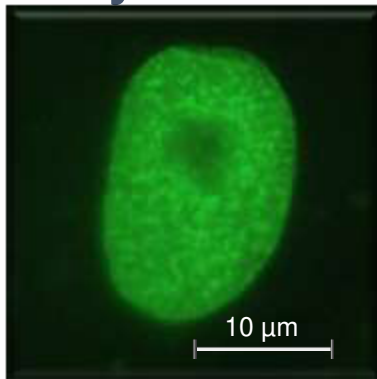
- No appropriate cell lines available
- Most of the culturable rodent cell lines are immortalised through hybridisation with cancer cells or are extracted from tumours

## The rationale for animal testing



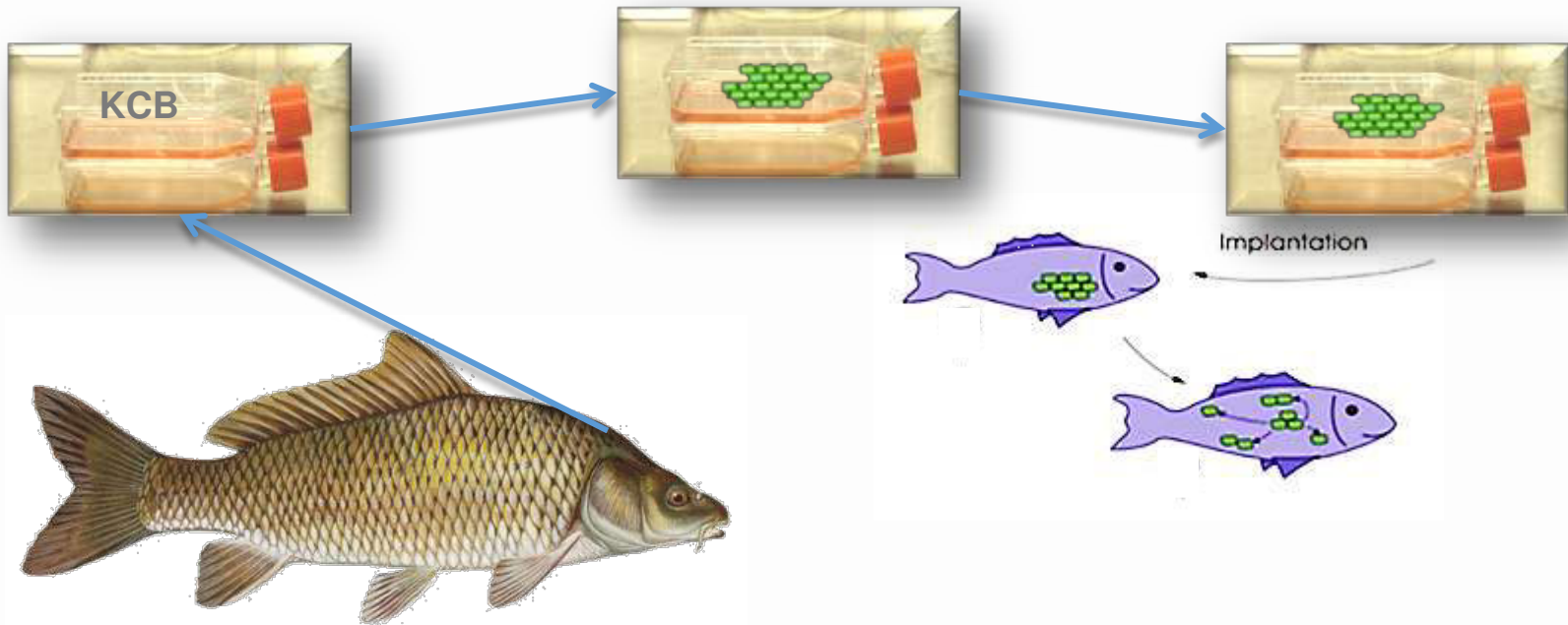
## Criteria for healthy stem cells

- 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

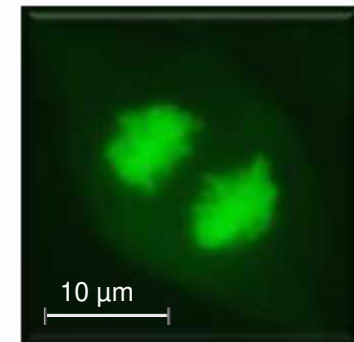
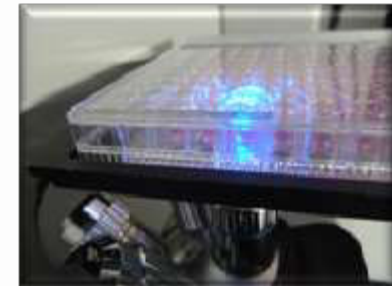
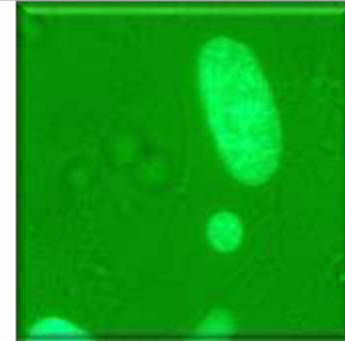
**KCB-GFP** = DSM ACC3285 Budapest treaty  
26°C growth temperature / no CO<sub>2</sub>





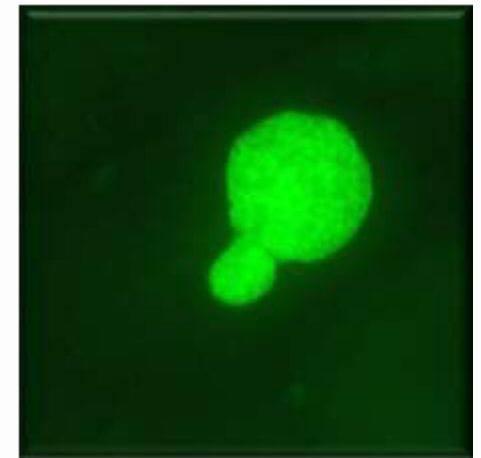
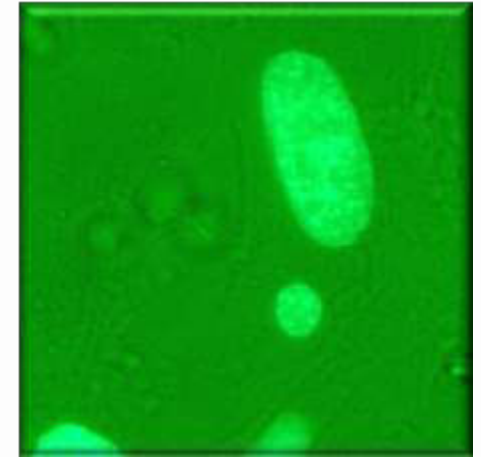
## Technical opportunities with the KCB cell line

- 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



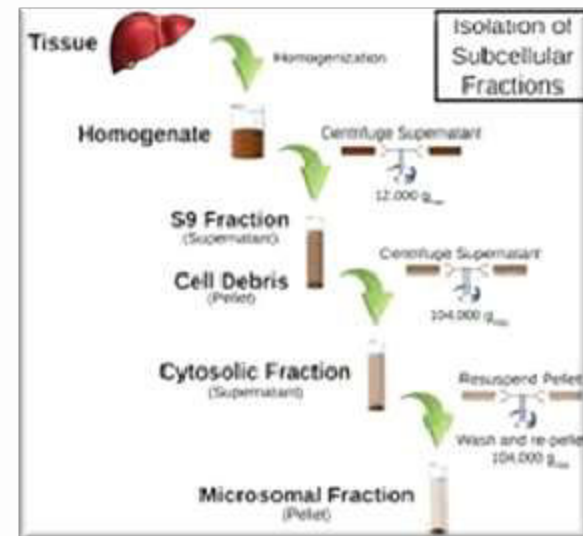
## The cancer initiating events

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!

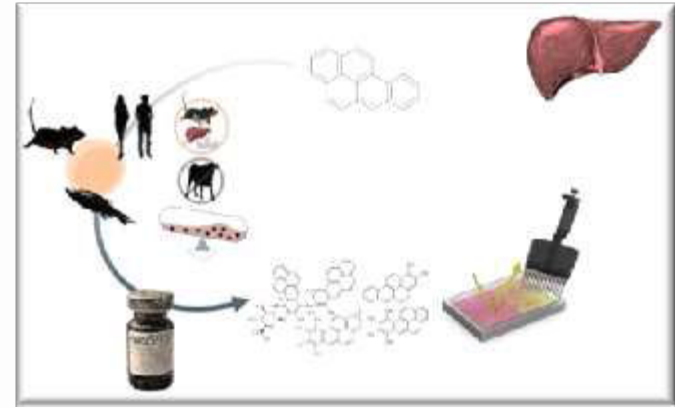
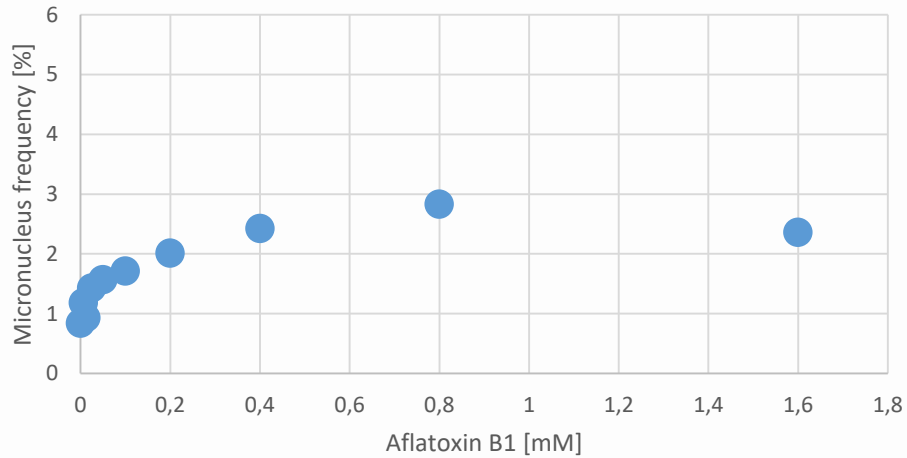


<https://www.xenotech.com>

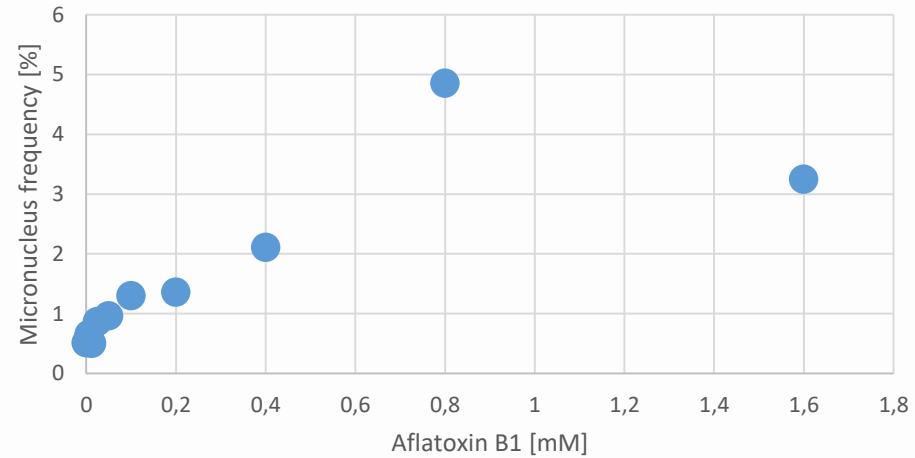
## MN Results



Aflatoxin B1 + S9



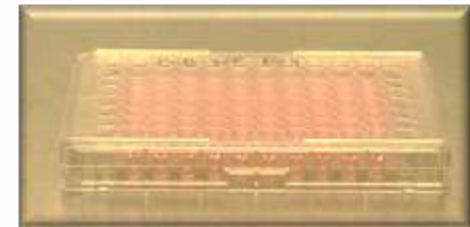
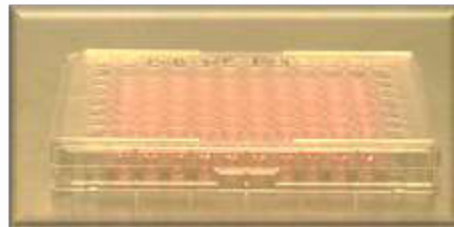
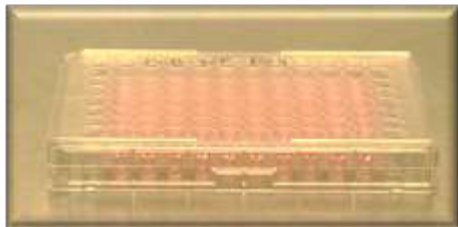
Aflatoxin B1 + EWOMIS S9



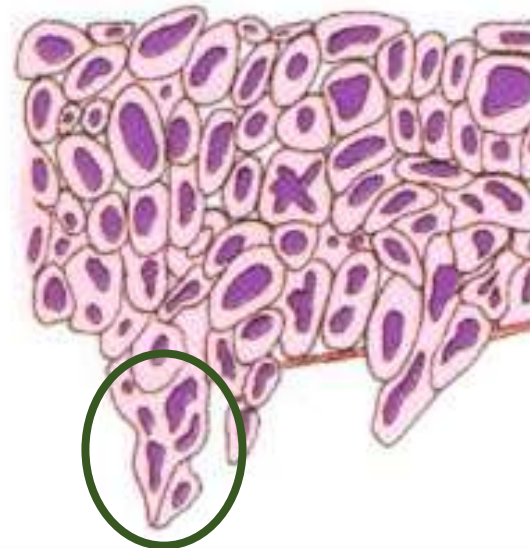
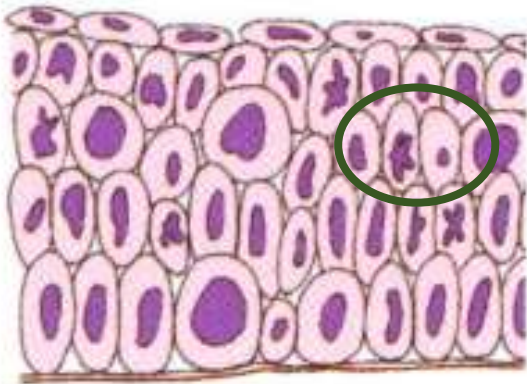
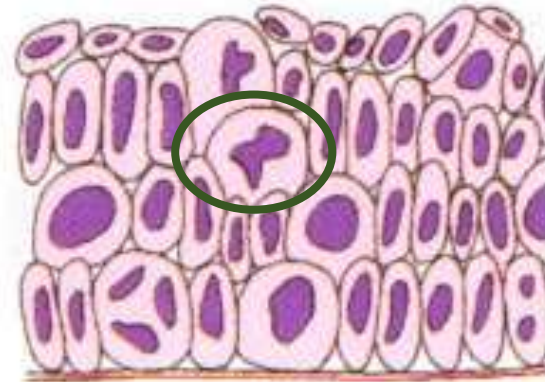
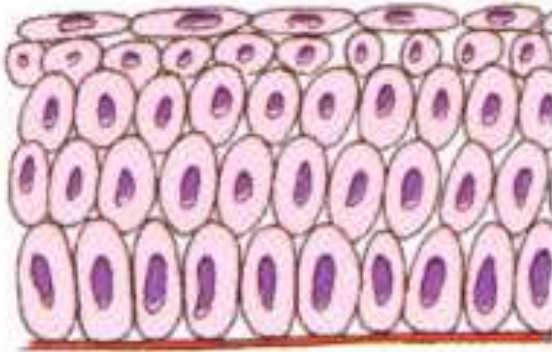
## Downstream-Analysis

- 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

MN positive

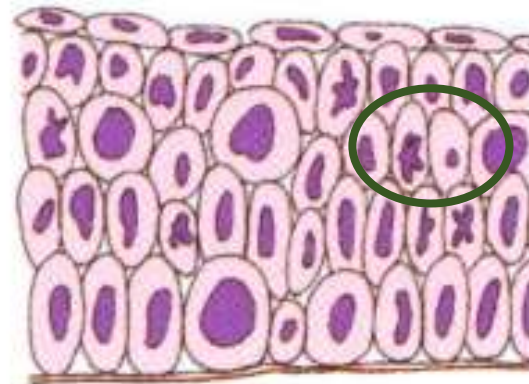
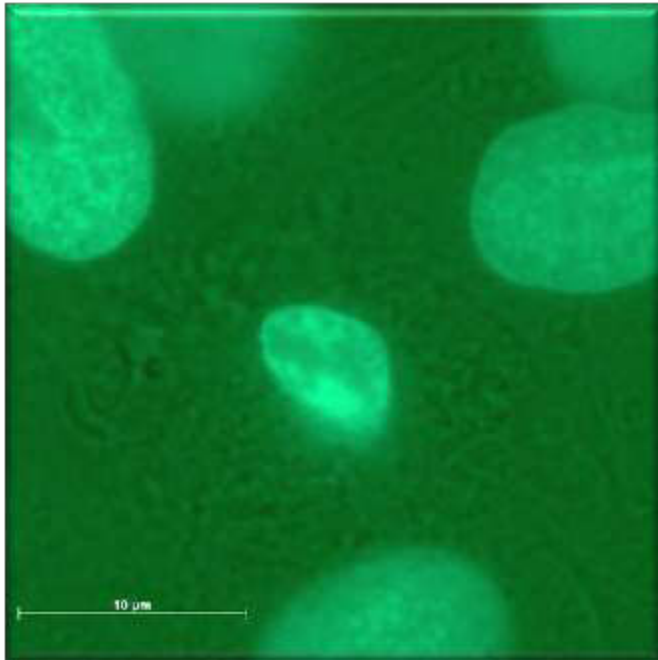


## Malignant degeneration

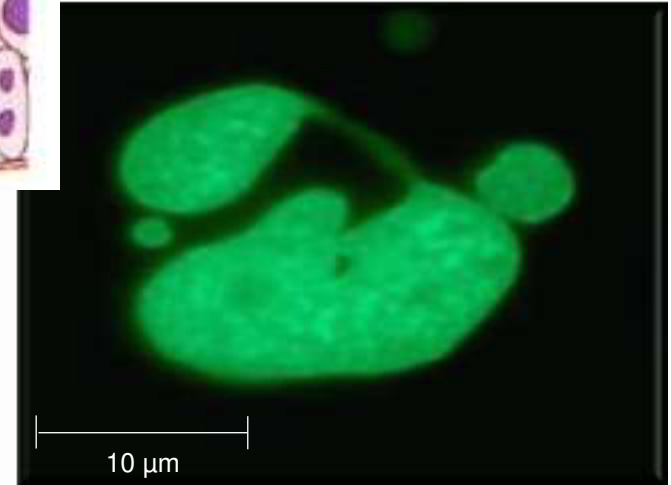
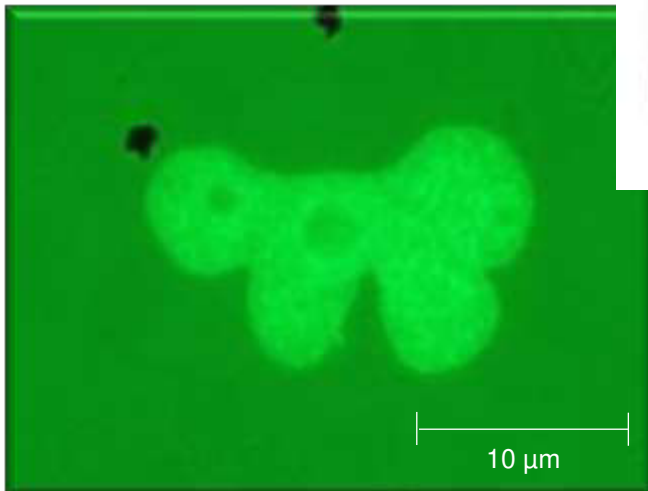
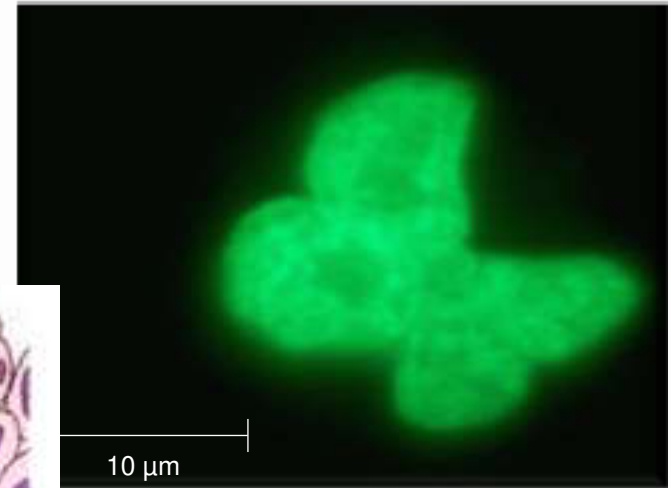
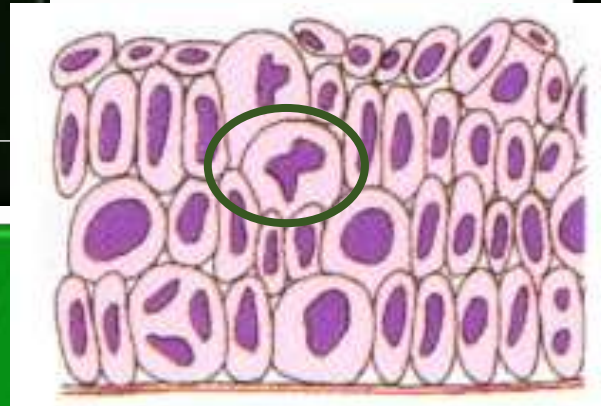
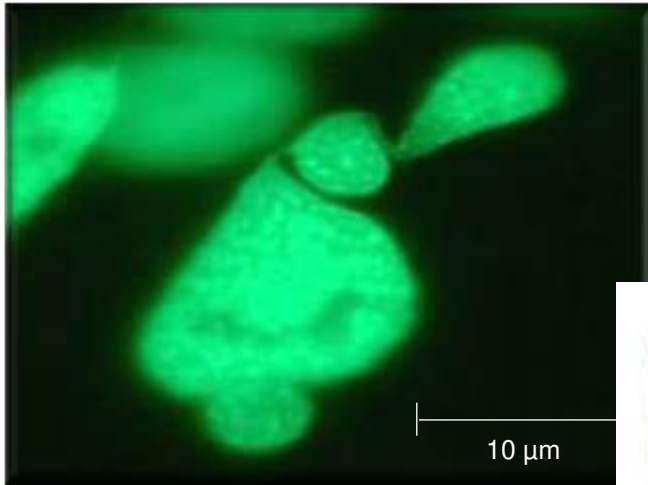


[https://www.ccc.ac.at/fileadmin/ccc/Uploads\\_Webseite/Die\\_Tumorzelle\\_Regele.pdf](https://www.ccc.ac.at/fileadmin/ccc/Uploads_Webseite/Die_Tumorzelle_Regele.pdf)

## Pyknototic and deformed nuclei

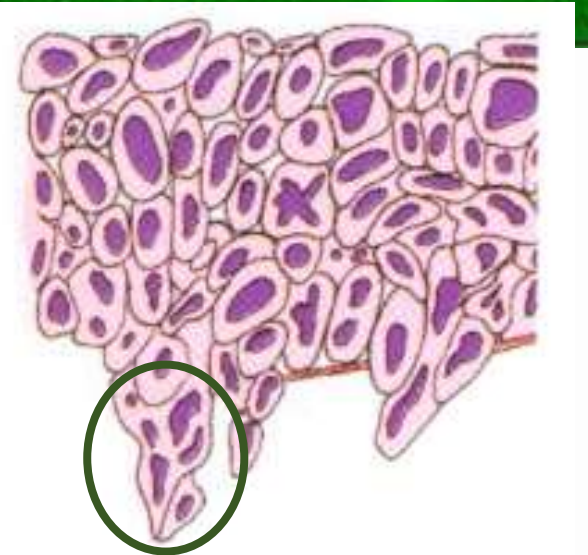
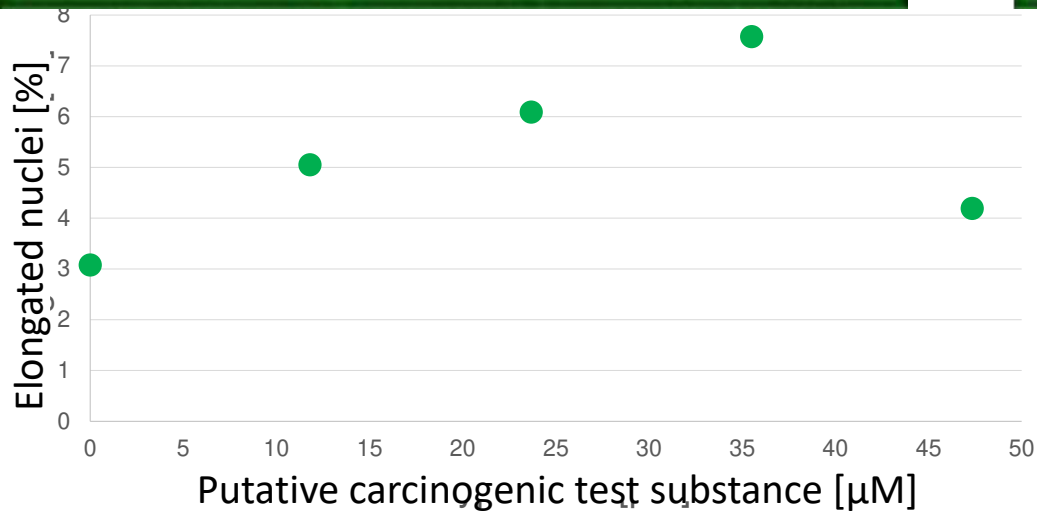
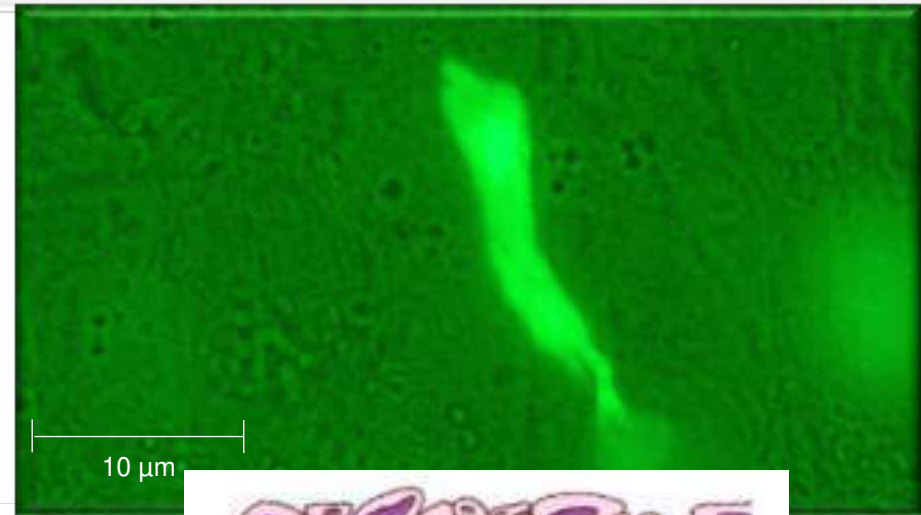
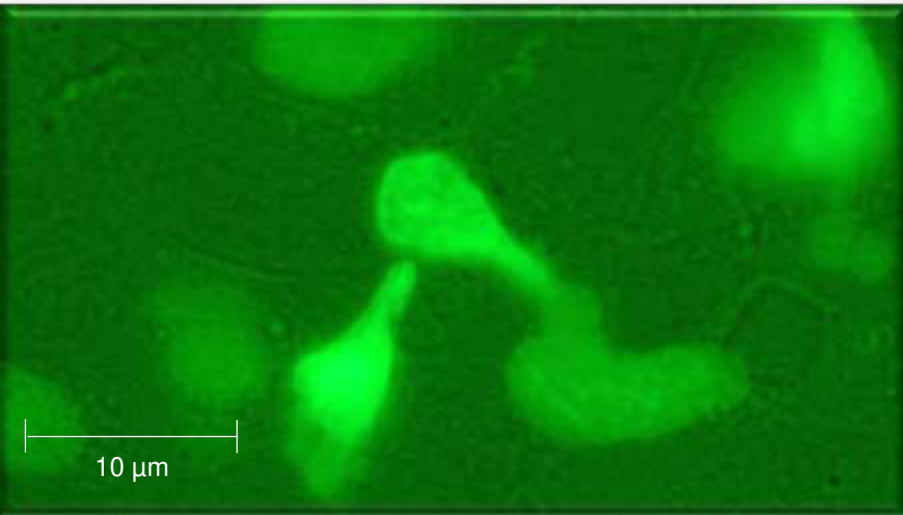


## Multi-lobed nuclei

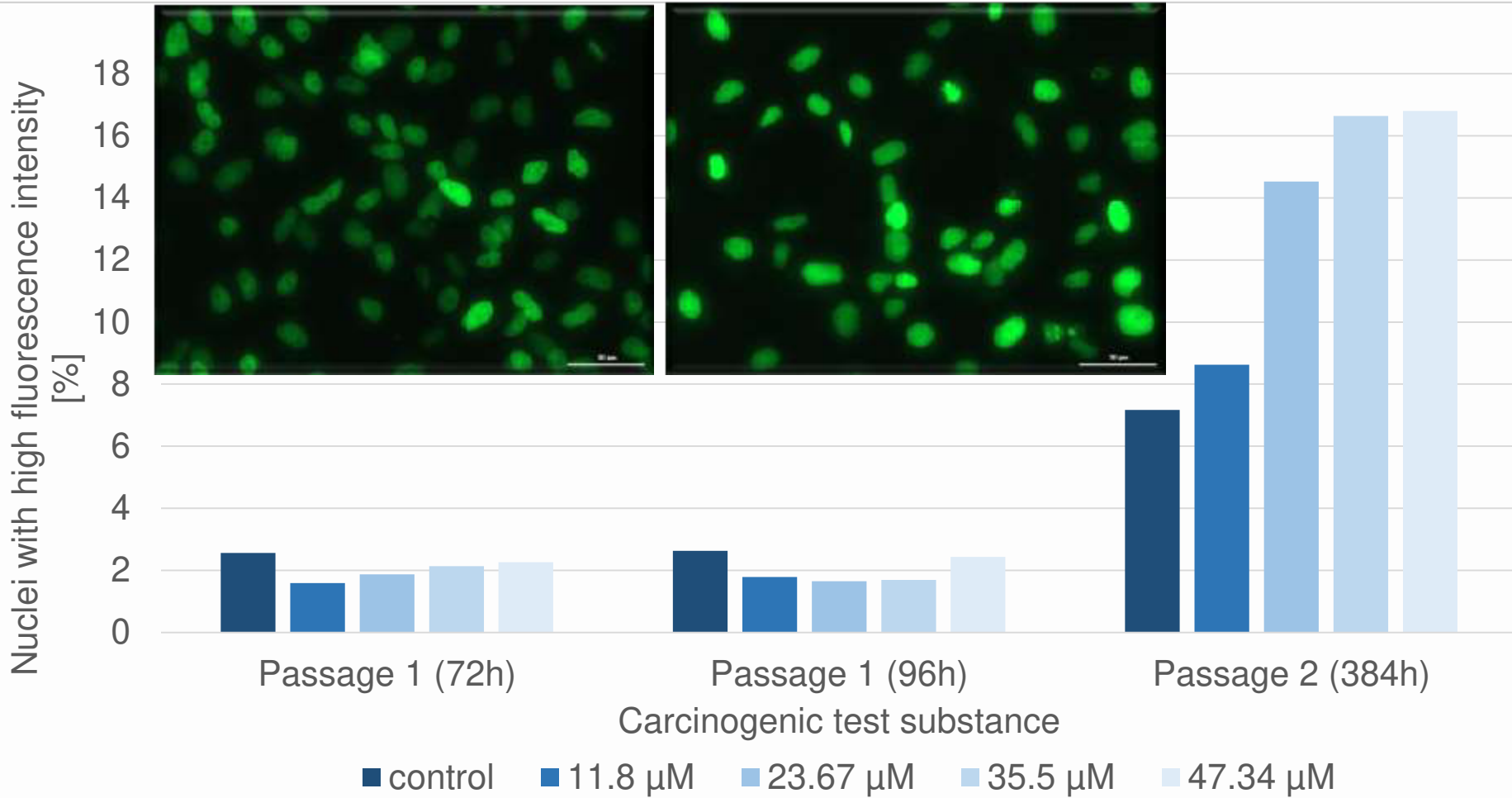




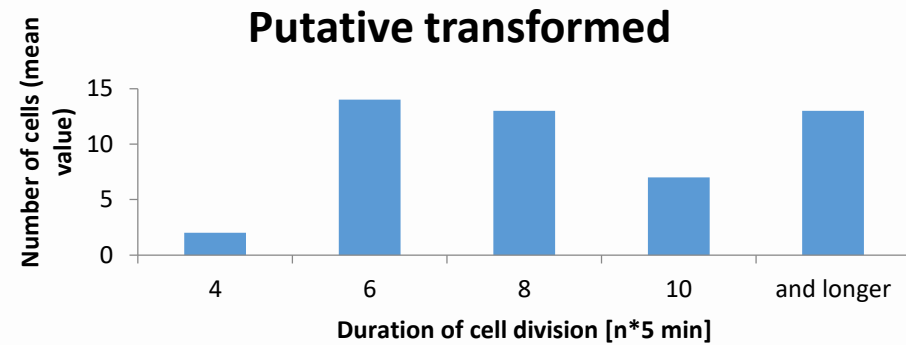
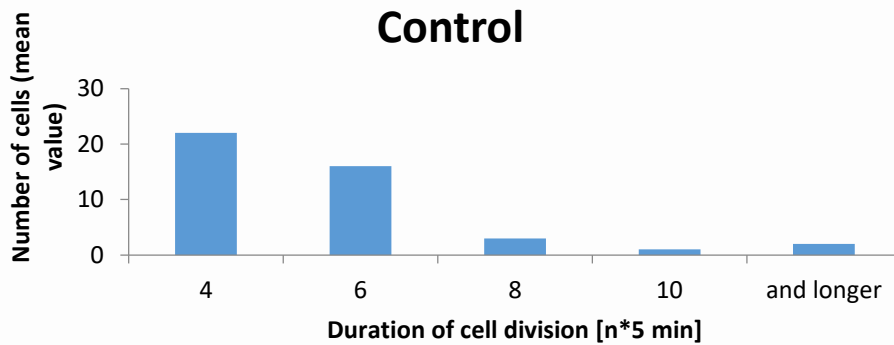
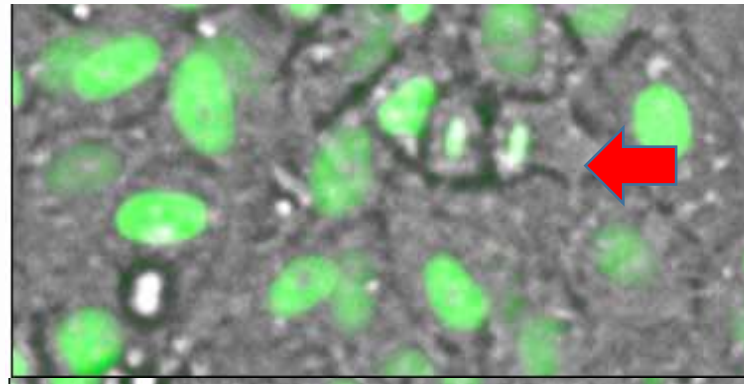
## Elongated nuclei



## Increase of fluorescence intensity

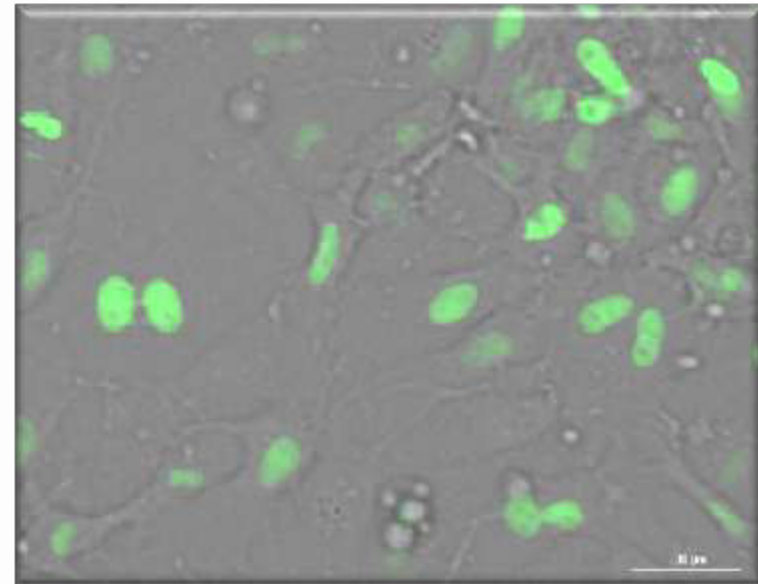


## Proliferation analysis



## Future perspectives and research needs

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



## Acknowledgement



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Thank you very much for your attention!

