Mouse models for the preclinical validation of immune therapies

Dr. Thomas Grunwald

SaxoCell Sparksmeeting

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Overview

Mouse models for the preclinical validation of immune therapies

Introduction

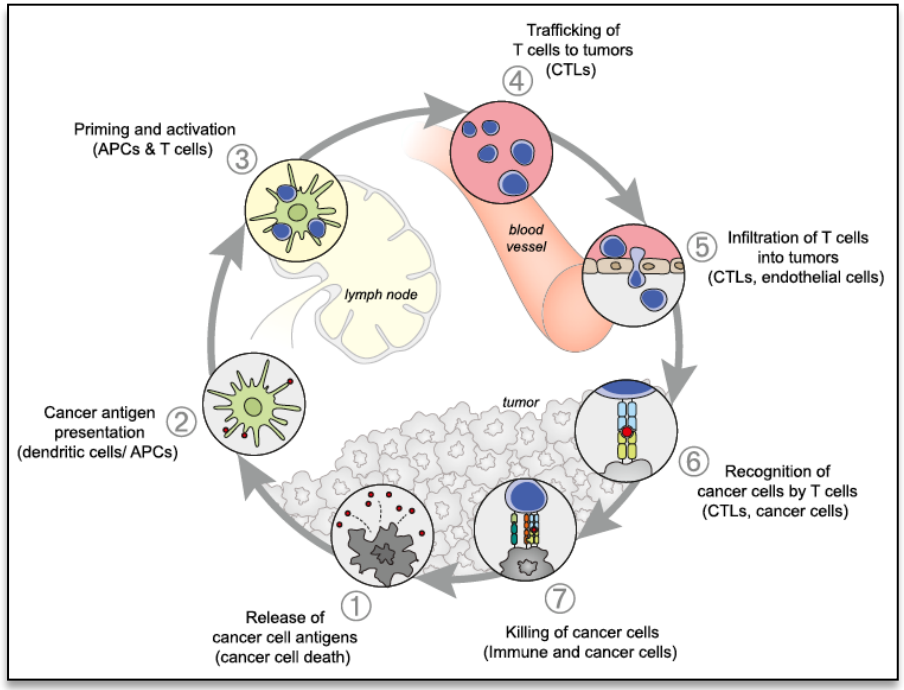
Research examples

- in vitro 3D cell culture system

- Tumor model establishment for the analyses of immune therapies / oncolytic viruses

Summary

Immune life cycle of tumors



Chen et al. Immunity 2013 39:1

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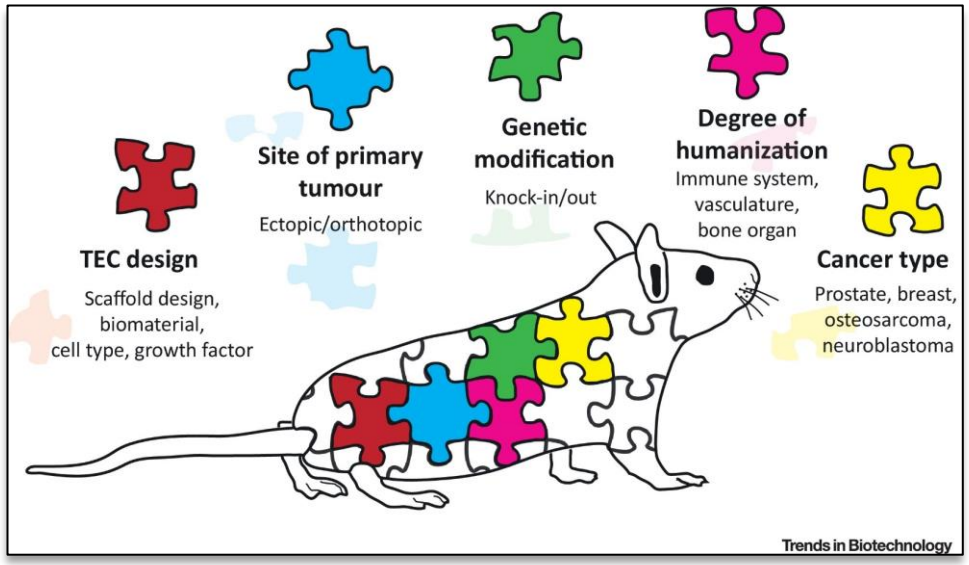
How to establish an in vivo tumor model

Questions before entering the preclincal phase

Choose the right models (including cancer type, read-out parameters, applications):

- **Syngenic models**
- **Cell-line derived xenograft models (CDX)**
- **Patient derived xenograft (PDX)**
- **Humanized mouse models (Hu-NSG, HIS)**
- **Ecotopic/ Orthotopic models**



Landgraf et al. Trends in Biotechnology 2018

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How to establish an in vivo tumor model

Read out parameter – most are available @ IZI

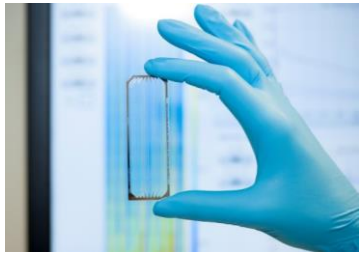
Bio-Imaging (e.g. MRI, PET, bioluminescence, ultrasound)

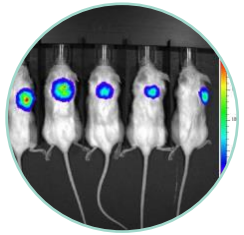
Cell quantification ex vivo (e.g. FACS, CyTOF, functional assays)

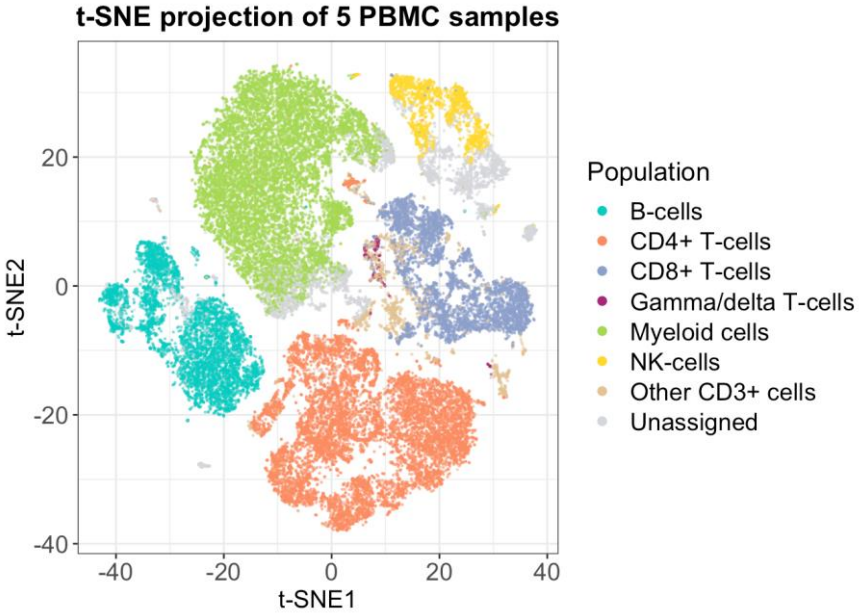
High-throughput technologies (cytokine bead-arrays, etc.)

Biomarkers, NGS-technologies, spatial Omic-technologies

Bio-informatics to analyse in depth of received data







https://biosurf.org/cytof_cytometrist.html

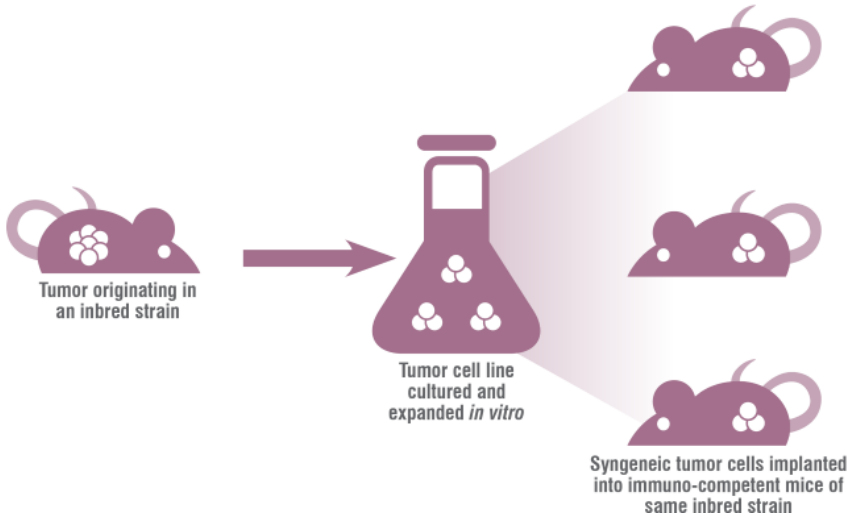
Selection of available animal models for immuntherapeutics

Pros and Cons of immunocompetent and immunodeficient mice

Immunocompetent mouse strain – syngenic mouse model

+ Analysis of impact of the immune system during tumor therapy

- Tumor cells and immune system not derived from human source

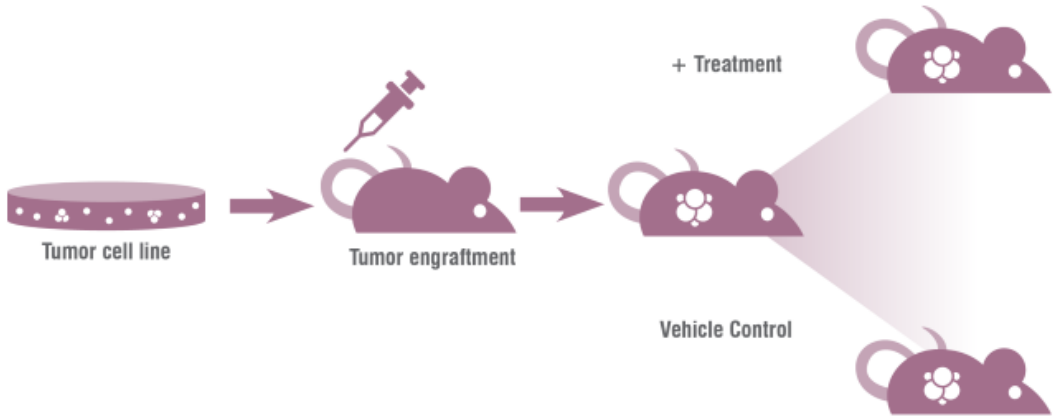


Immunodeficient mouse strains

+ Use of human tumor cells for analyses in regard to the therapy option

+ Longitudinal studies with tumor volumes and genetic modified reporter cell lines

- Immune system transferred experimentally



• Pictures by criver.com.

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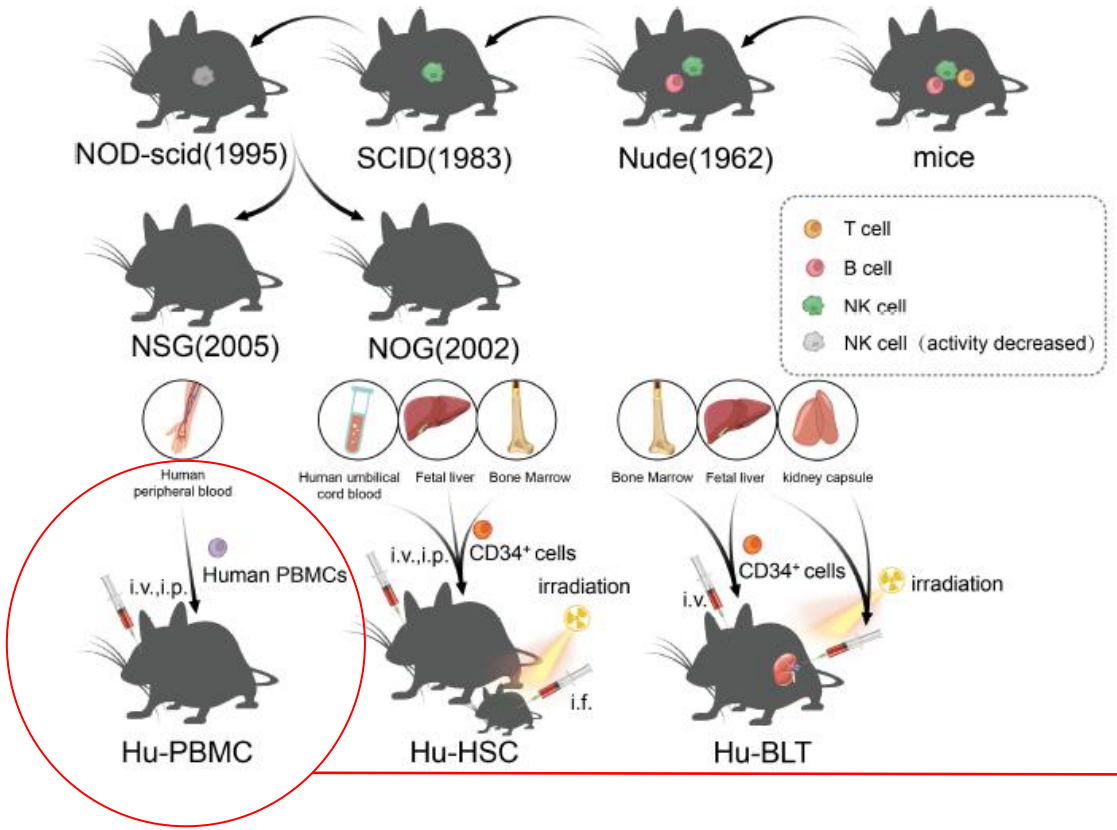
Establishment of the humanized mouse models

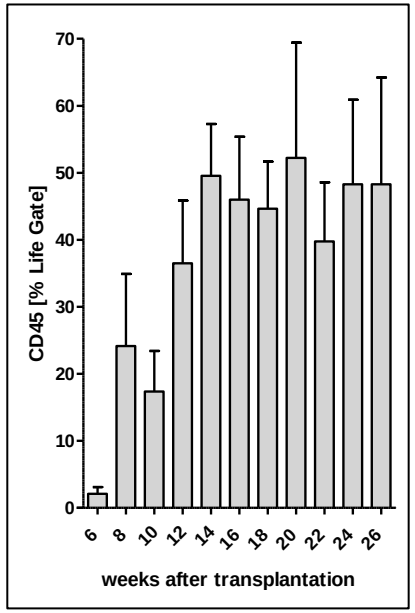
History of famous immunodeficient mouse strains



Hu-Mice is used for different tumor models:

Cell derived tumor models (CDX)

Patient derived tumor models (PDX)



- Picture by Zhou et al, 2023
- Data by Scholbach J, et al., PLoS One. 2012;7(10):e46772.

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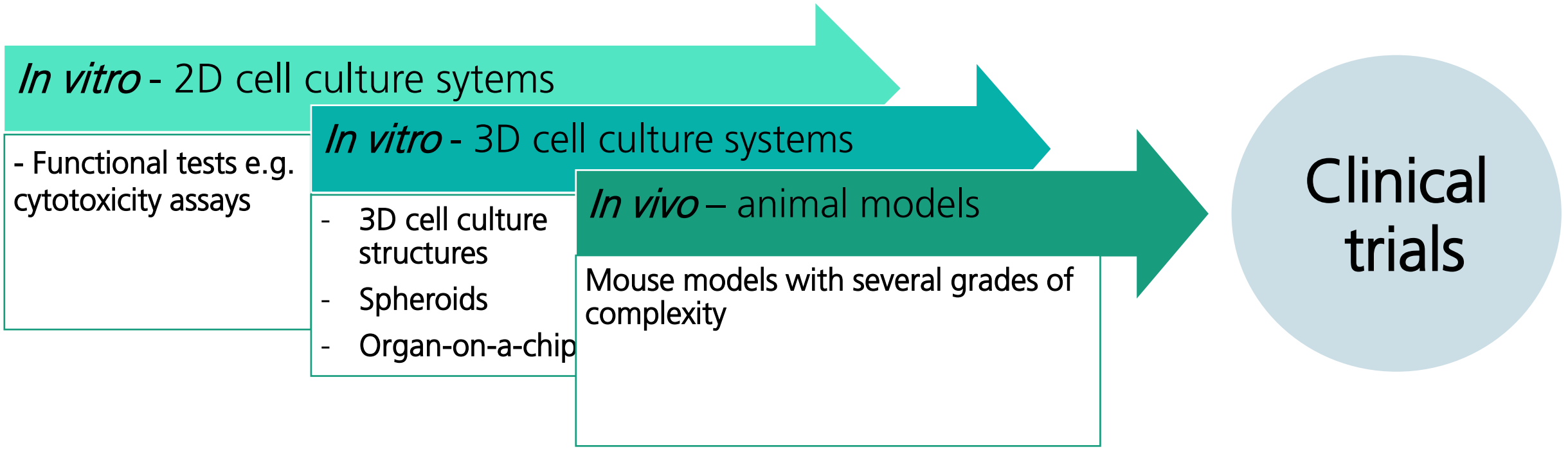
Overview on different translational test systems



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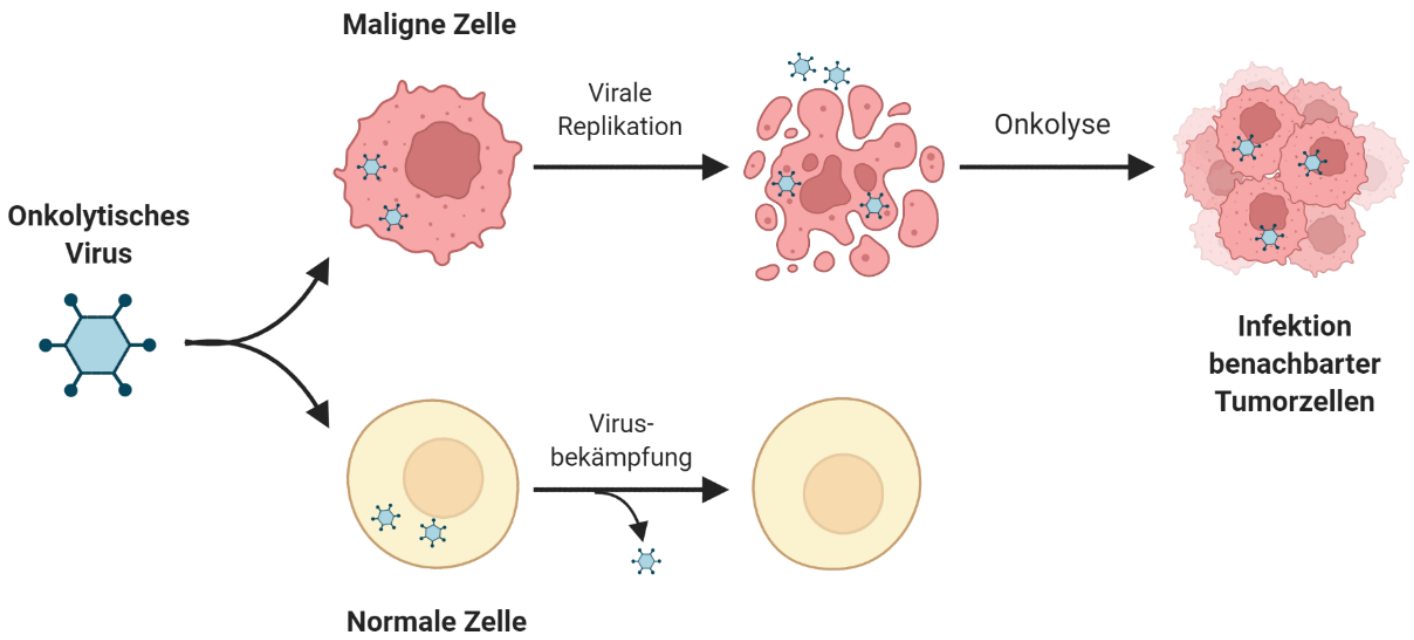
Option of tumor therapy by oncolytic viruses

Tumor regression was observed after virus infection previously



THE INFLUENCE OF COMPLICATING DISEASES UPON LEUKÆMIA.*

By George Dock, A.M., M.D.,

Professor of Medicine in the University of Michigan, Ann Arbor, Michigan.

- 42 years old women with myeloid leukemia
- Influenza-infection
- Amount of leukocytes was reduced by 70-fold
- Reduction of liver and spleen size
- Leukemia was undetectable

REMISSIONS IN LEUKEMIA OF CHILDHOOD FOLLOWING ACUTE INFECTIOUS DISEASE

Staphylococcus and Streptococcus, Varicella, and Feline Panleukopenia

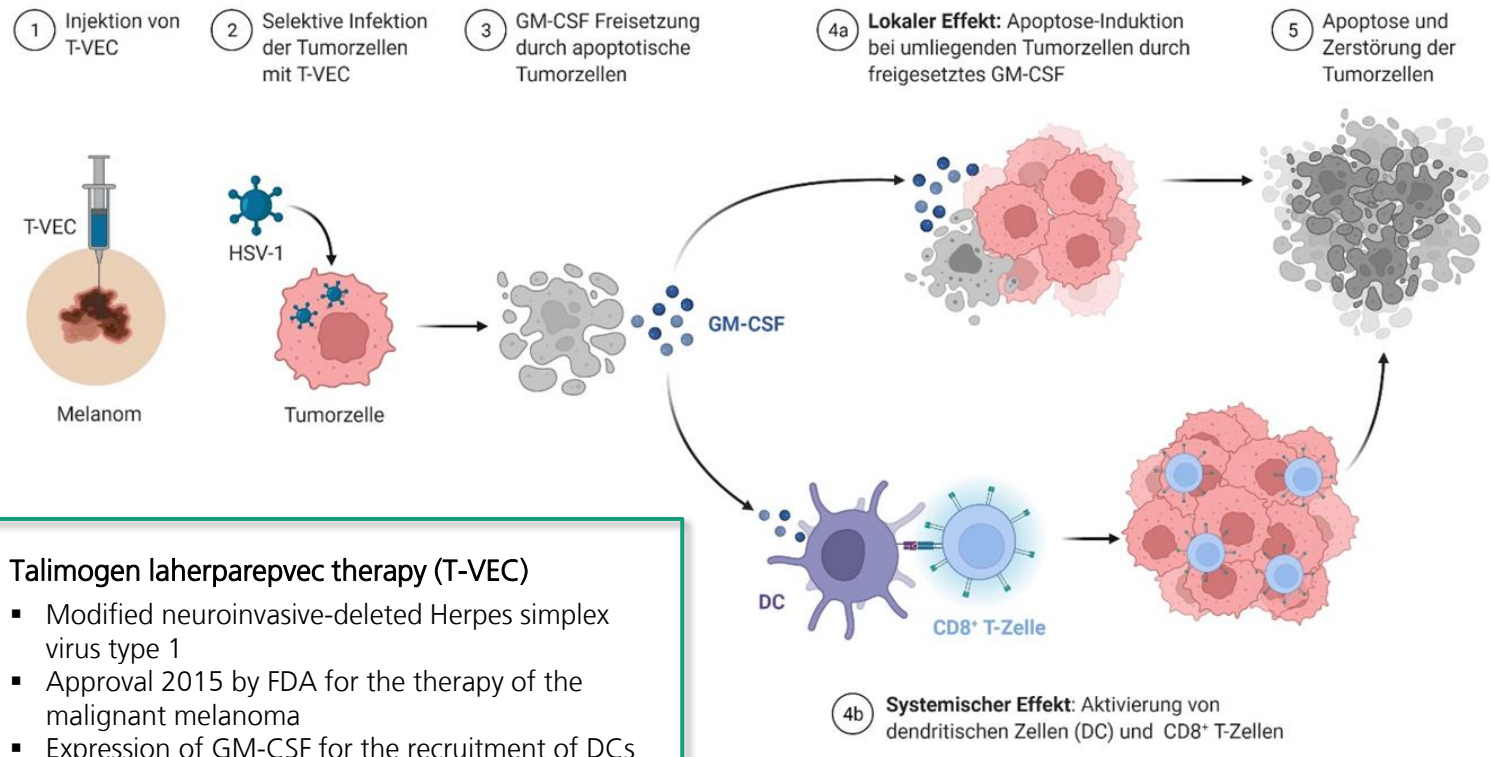
Howard R. Bierman, M.D., D. Michael Crile, M.D., Kenneth S. Dod, M.D., Keith H. Kelly, M.D., Nicholas L. Petrakis, M.D., Laurens P. White, M.D., and Michael B. Shimkin, M.D.

- Child 18 month old with lymphatic leukemia
- Varizella-Zoster-Virus + bacterial infection
- 10-fold reduction of leukocytes
- Reduction of liver and spleen size

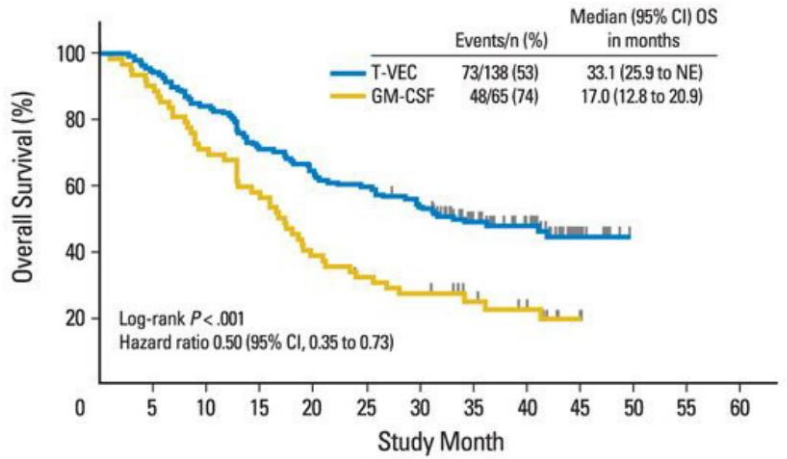
- Pictures modified with BioRender from Groeneveldt, C. et al. (2020) Immunotherapeutic Potential of TGF-β Inhibition and Oncolytic Viruses. Trends in Immunology.
- Dock, G. (1904) The influence of complicating diseases upon leukaemia. The American Journal of the Medical Sciences.
- Bierman, H.R. (1953) Remissions in leukemia of childhood following acute infectious disease […]. Cancer.

Oncolytic Viruses

Modified Herpesviruses: T-VEC (Imlygic®) – intratumoral injection in humans

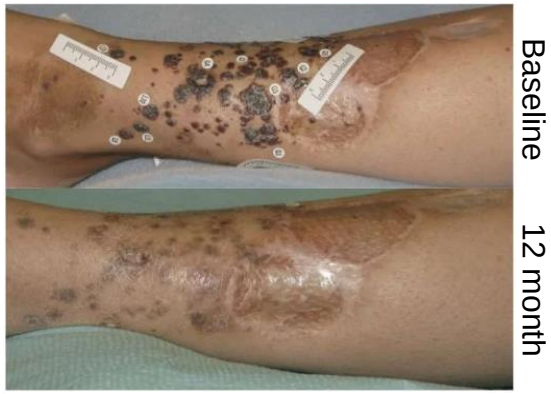


Survival with T-VEC as first line therapy



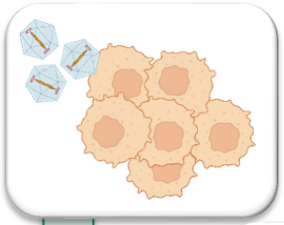
Talimogen laherparepvec therapy (T-VEC)

- Modified neuroinvasive-deleted Herpes simplex virus type 1
- Approval 2015 by FDA for the therapy of the malignant melanoma
- Expression of GM-CSF for the recruitment of DCs and macrophages
- Immune activation and tumor regression also in distant tumors

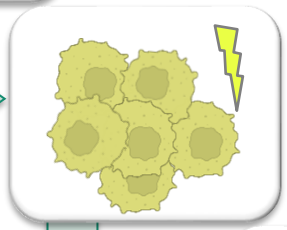


- OV: Onkolytic virus
- Andtbacka, R.H.I. et al. (2019) OPTiM: a randomized phase III trial of talimogene laherparepvec. ImmunoTherapy of Cancer
- Pictures modified with BioRender from Johnson D.B. et al. (2015) Talimogene laherparepvec (T-VEC) for the treatment of advanced melanoma. Immunotherapy.

Example of testing an HSV-based oncolytic virus vector



Lentiviral gene transfer in NSCLC-tumor cell line



Luciferase-expressing reporter tumor cells

s.cu. Injection of reporter-tumor cell in NSG mice

Intratumoral therapie mit oncolytic HSV

Tumor progression and detection of lytic processes

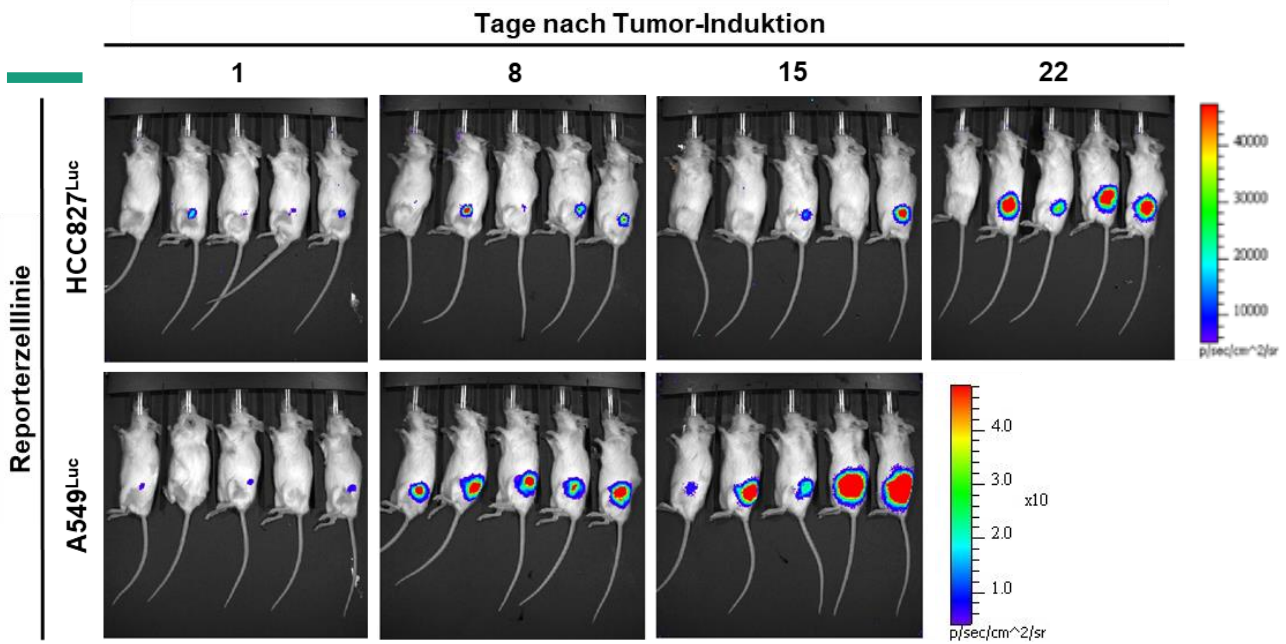
Lung carcinoma

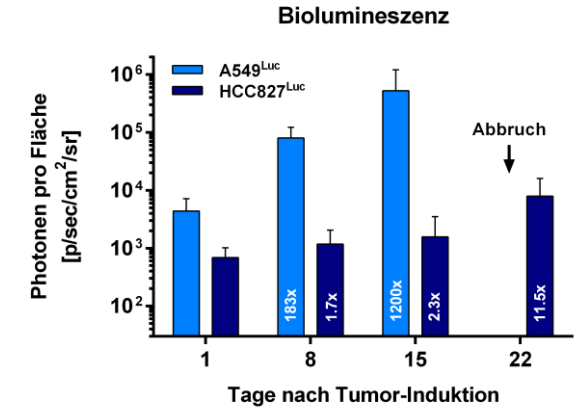
- Very malignat tumor entity
- 5-year-survival: 15.6%
- Risk factors: smoking, air pollution, genetic factors
- State of the art therapy: resection, chemo- and/or radiotherapy
- Rate of remission: 30% - 55%

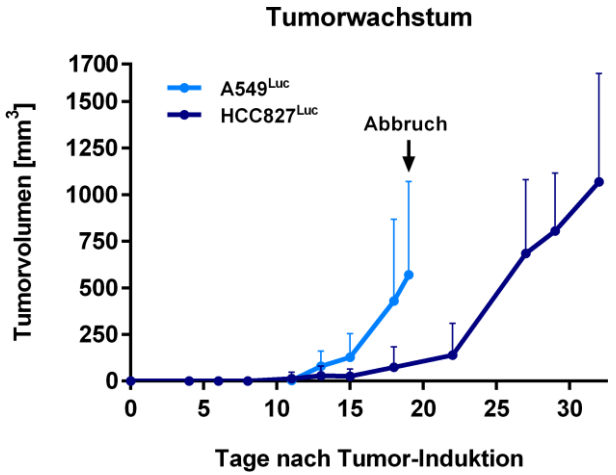
- NSCLC: *non-small-cell lung cancer* | s.cu.: sub cutanous | NSG: immunodeficient NOD scid gamma mouse

Created with BioRender.com

HSV-based oncolytic virus vector







- Subcutanous application of 2 x 10^5 of indicated reporter tumor cells in NSG mice
- Determination of tumor mass 2-3x per week
- Measurements of bioluminescence 1x per week

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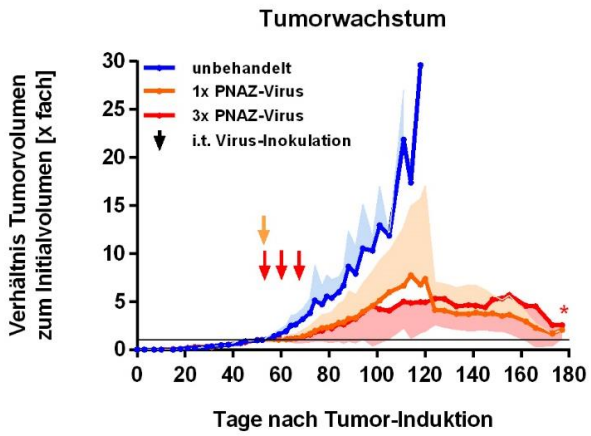
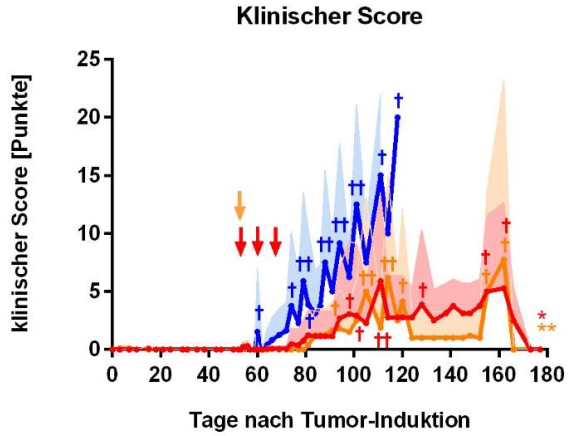
Research with HSV-based oncolytic Virus Vector

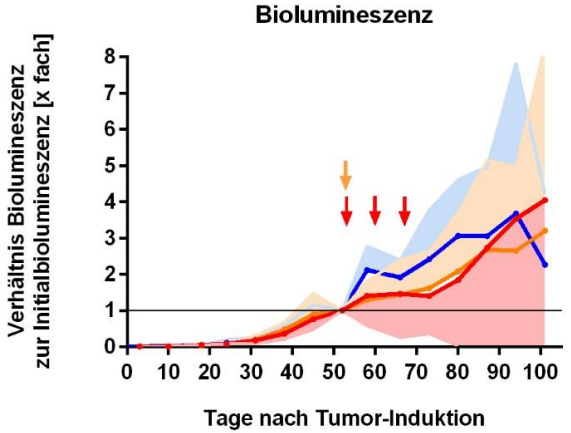
PNAZ-Virus:

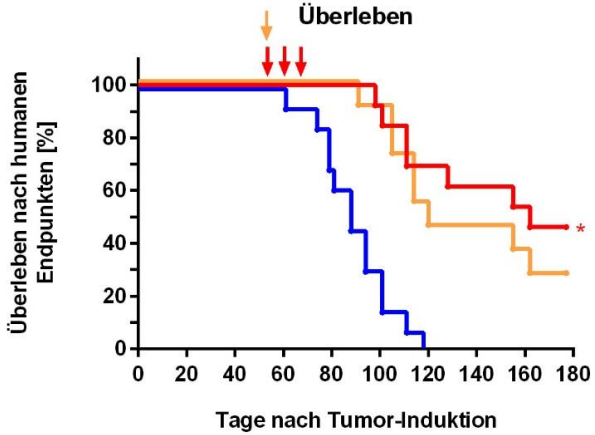
- Deletion of neuroinvasion
- Attenuation
- EGFR Tropism

- Injection of tumor cells: 2 x 10^5 $HCC827^{Luc}$
- OV application intratumoral: 3,3 x 10^6 PFU PNAZ-virus day 53 (day 60, day 67)
- Determination of tumor volumen and bioluminescence 2x per week
- Survival and clinical score









- †: Euthanasia according human end points (score ≥ 20)
- n = 13, n_{1xPNAZ} = 11 | Statistik: Kruskal-Wallis-Test, *: p ≤ 0,05; **: p ≤ 0,01

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Summary

Several in vivo models are available to monitor cancer therapies.

Establishment of a human immune system in mice is still not perfect – further improvement needed to simulate complex immune processes. Immunodeficient PDX-models and humanized mice models are widely used.

Complex 3D in vitro systems are still missing to substitue in vivo models.

Acknowledgments

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and to our partners from Affimed

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Thank you for your attention!

CONTACT
Dr. Thomas Grunwald
Department of Vaccines and Infection Models
Preclinical Validation Unit
Tel. +49 341 35536-5423
Thomas.grunwald@izi.fraunhofer.de

Fraunhofer-Institut für Zelltherapie und Immunologie IZI
Perlickstraße 1
04103 Leipzig
www.izi.fraunhofer.de

Humanized mouse models

Immundeficient strains besides NSG/NXG

TABLE 1 | Humanized mice to study ILC-cancer interactions.

Mouse Model	Human Tumor Cells Administered	Human Cells Engrafted	Human Lineage Reconstitution (*lineages with improved reconstitution compared to NSG mice)	References
MISTRG	Me275 melanoma cells	CD34+ HSPCs	*monocytes, *macrophages, *DCs, T, B and *NK cells	(21)
SRG-15	Raji tumor cells and K562 tumor cells	CD34+ HSPCs	myeloid cells, *T, B *NK cells and *ILCs	(68)
NOG-IL-15 Tg	NCI-N87 human gastric cancer cell	Peripheral blood NK cells and *in vitro*-expanded NK cells	*NK cells	(69)
hIL-7xhIL-15 KI	-	CD34+ HSPCs	T and *NK cells	(70)
BRGSF	-	CD34+ HSPCs	*myeloid cells, T, B, DC, *NK cells and *ILCs	(71)
O-PDX (MISTRG)	Neuroblastoma	CD34+ HSPCs	*NK cells	(72)
Hu-PDX (NSG)	Lung adenocarcinoma	CD34+ HSPCs	T, B and NK cells	(73)
HTM (NSG)	Breast cancer	CD34+ HSPCs	T, B, NK cells and macrophages	(74)

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