Onkološki Inštitut
Institute of Oncology
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Mišji tumorski modeli

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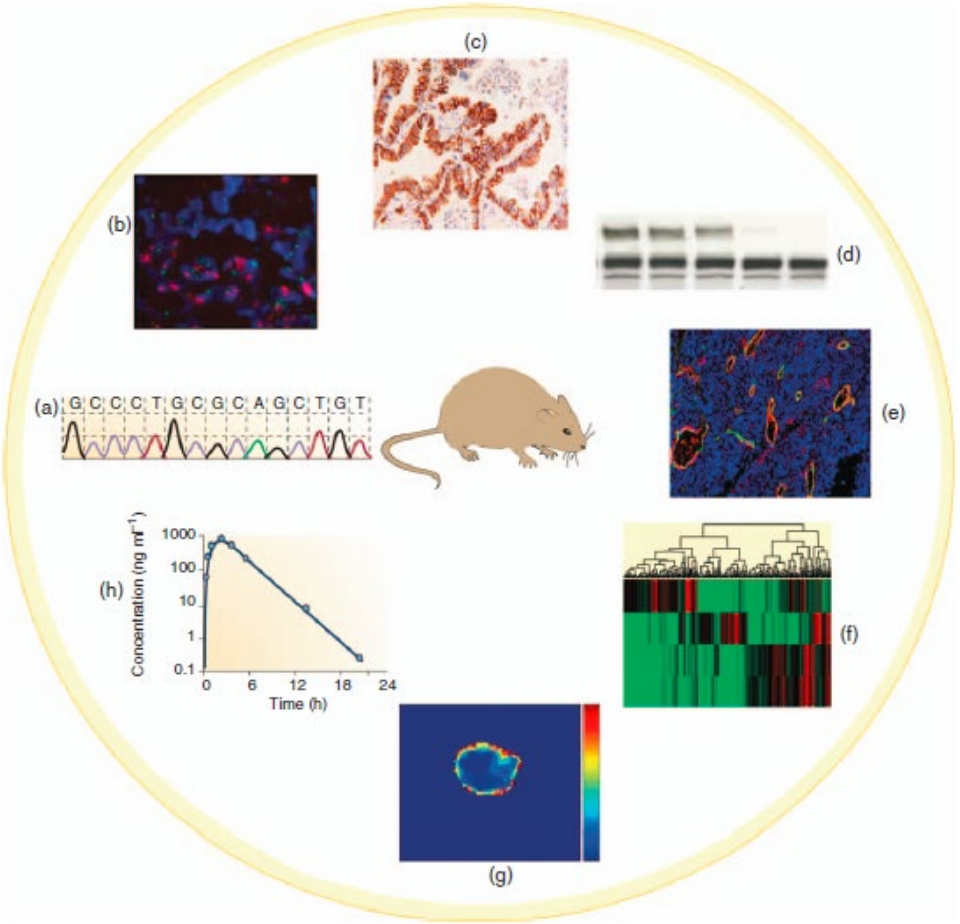
SDLŽ, 11.11.2025, on -line

Za delo v predklinični ali eksperimentalni onkologiji so potrebni:

- In vitro modeli:
  - Genomika in proteomika
  - Celične kulture
- Matematični modeli
- Ex vivo modeli:
  - Organoidi
- In vivo modeli:
  - Tumorski modeli
  - Živalski modeli



- Študij biologije nastanka raka
- Preizkušanje različnih zvrsti zdravljenja raka
- Testiranje strategij za prevencijo nastanka raka
- Razvijanje postopkov za zgodnje odkrivanje in diagnozo raka

Področja raziskav v onkologiji

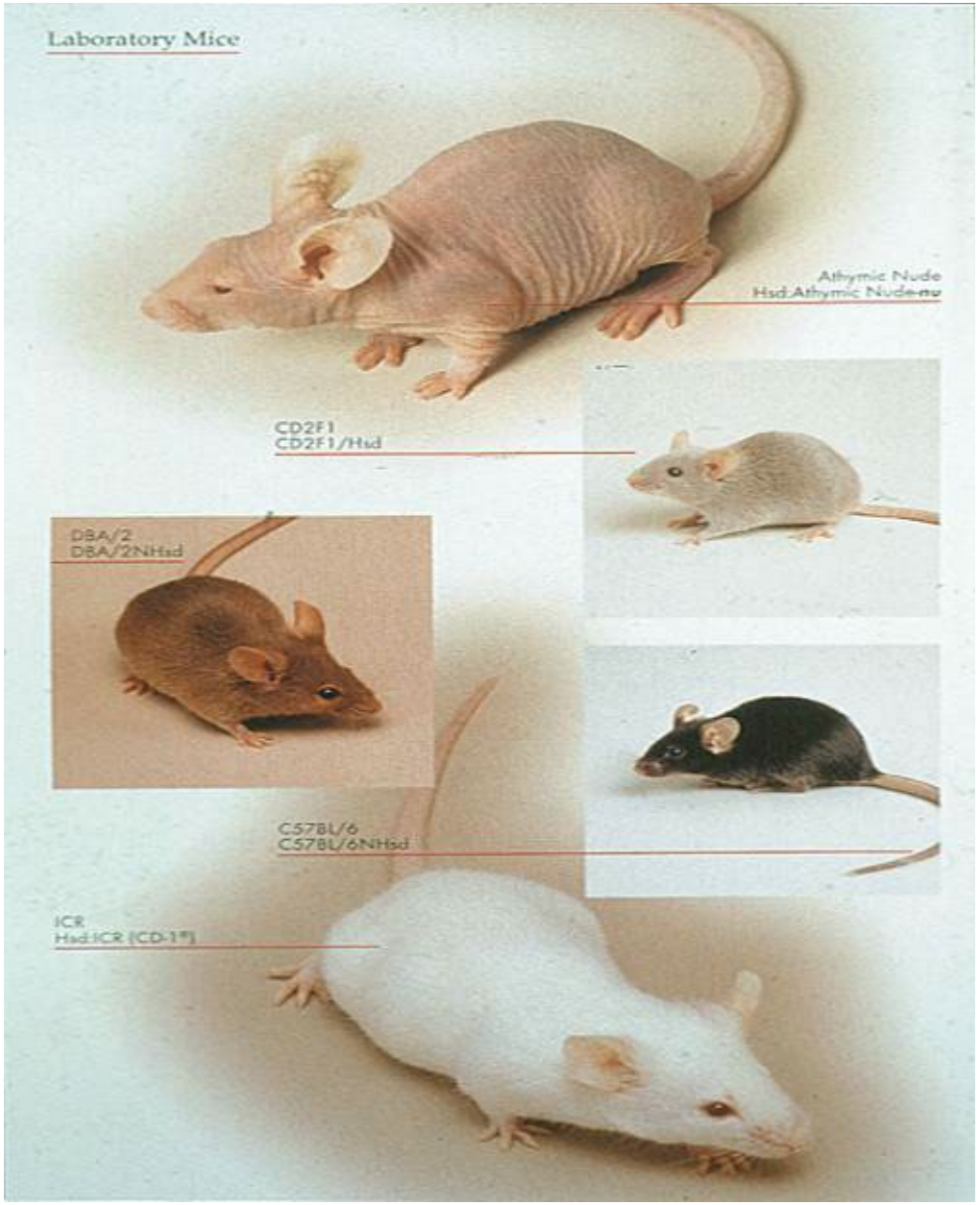
- Tumorska biologija
  - Specifične lastnosti tumorskih celic
  - Interakcije med tumorjem in gostiteljem
- Testiranje in prenašanje novih zdravljenj v klinično prakso

Kaj je dober model?

- Celične kulture
- Sferoidi/organoidi/organi na čipih…
- Glodalski tumorski modeli
- Humani tumorski ksenografti

- Samo orodja, ki se uporabljajo za študij specifičnih vprašanj v zvezi s humanimi tumorji

Laboratorijske miši



Prednosti in slabosti uporabe mišjih tumorskih modelov

Advantages	Limitations
Small size, easy to handle and take care of, and short-tumor generation time.	Mice are very different, in terms of size, life span, organ morphology, and physiology and thus differ in drug PK and PD from human beings.
Cheaper than other animal models of cancer, allowing the use of large numbers for statistical measurements.	One critical difference in the mouse is the activity of the telomerase enzyme, which is largely inactive in adult human cells. (Mouse cells transform more readily and thus require fewer genetic alterations for malignant transformation.)
High tumor incidence and relatively rapid tumor growth.	Mouse models tend to develop relatively few metastases or display metastases with different tissue specificity as compared with human tumors.
Many mice can be treated at the same time to observe dose responses.	Differences in metabolic rate and pathways might result in a different drug response in mouse models (e.g., the cytochrome P450 pathway for drug metabolism).
Genetically, the best characterized of all mammals used in cancer research.	Due to a limited number of initiating genetic alterations, mouse tumors are typically more homogeneous, and this can be an obstacle to modeling the heterogeneity of human cancers.

Animal Biotechnology.
DOI: https://doi.org/10.1016/B978-0-12-811710-1.00005-7

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Miši, ki se uporabljajo v biomedicinskih raziskavah

- Inbridirane imunsko odzivne živali
- Gole (brez T limfocitov)
- SCID (brez B in T limfocitov)
- Transgene
- Knockout
- Humanizirane

Inbridirane imunsko odzivne živali

C57BL/6 miš
Clarence Cook Little, 1920
ustanovitelj Jackson laboratory
Sekveniranje genoma 2002

- Prednosti
  - Relativno poceni
  - Veliko število ustanovljenih tumorskih modelov
  - Različna mesta injiciranja
- Slabosti
  - „imunost“
  - Genska raznolikost

TABLE 5.2 Criteria for choosing the optimum inbred mouse model.

Biologic:

1. Multistage: the model shows clear evidence of progression, enabling analysis of genetic and biological events associated with these transitions.
2. Tumor histology and pathology are similar to equivalent human disease.
3. Tumors metastasize from primary site.
4. Model has specific properties that make it suitable for studies of one of the following:
   - Angiogenesis
   - Apoptosis/survival
   - Tissue invasion
   - Role of the immune system

Genetic:

1. Multiple mutations in specific genes, at least some of which reflect those of equivalent human tumors.
2. Gross chromosomal changes (LOH, deletions, recombinations) in regions orthologous to those altered in human disease.
3. Tumors bear specific alterations in one or more pathways known to be involved in human cancer, for example, p53.
4. Gene expression profiles similar in mouse and human tissues.
5. Tumor shows strong effects of genetic background, enabling studies of genetic susceptibility

Etiologic:

Tumor model reflects exposure to environmental factors known to play a causal role in human tumor induction, for example: Environmental mutagens like chemical, radiation.

Therapeutic:

1. The model is suitable for the testing of new *therapies*:
   - *Chemicals*
   - Viral vectors
   - Immunological targets.
2. The model is predictive of human clinical trial results.

DOI: https://doi.org/10.1016/B978-0-12-811710-1.00005-7

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Gole miši

- Prednosti
  - Dobra karakterizicija, siroka uporaba
  - Odsotnost dlak omogoča dobro vizualizacijo podkožnih tumorjev
  - Dovolj imunsko oslabljene, da je mogoča rast mnogih humanih celic in tumorjev
  - Na voljo na različnih genetskih osnovah
- Slabosti
  - Prisotne nekaj ekstratimusne T celične funkcije
  - Prisotni B limfociti
  - Normalno število makrofagov, NK celic, normalna funkcija APC celic in normalna funkcija komplementa – NARAVNA ODPORNOST

SCID miši

Fox Chase SCID® Beige Mouse

A congenic mouse that possesses both autosomal recessive mutations SCID (*Prkdc^{scid}) and beige (Lyst^{bg}).* The SCID mutation results in severe combined immunodeficiency affecting both the B and T lymphocytes. The beige mutation results in defective natural killer (NK) cells.

Prednosti

- Boljše kot gole miši – manj spontanih regresij tumorjev, večji procent humanih tumorjev, ki jih lahko gojimo na SCID miših
- Na voljo na različnih genetskih osnovah

Slabosti

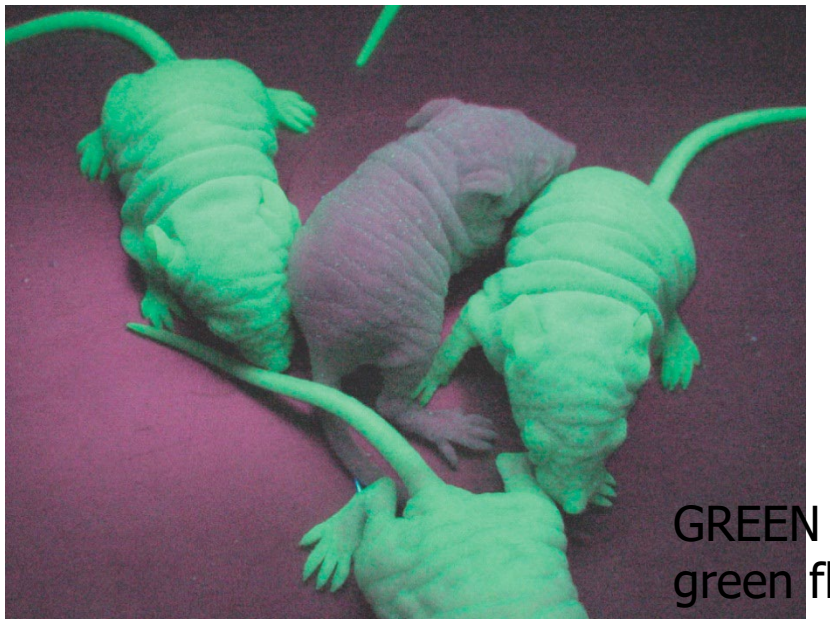
- V odvisnosti od vrste in okolja obstaja nekaj produkcije T in B limfocitov po 12 tednu starosti
- Normalno število makrofagov, NK celic, normalno delovanje APC , pri nekaterih je funkcija komplementa povečana
- Visoka incidenca limfomov timusa – krajša življenjska doba
- Radioobčutljive zaradi napak v DNA popravljalnih mehanizmih

Gensko spremenjene miši – transgene miši

- Netransgene miši: spontane mutacije, kemično inducirane, drugi vir nastanka (npr. sevanje)
- Transgene:
  - Knock-in
  - Knock-out
  - Usmerjene mutacije (Crispr/Cas9)

Transgene miši – vnos tujega gena –knock-in miši

- Prednosti
  - Tumorji se razvijejo spontano in v “naravnem” organu
  - Tumorji imajo naravno hitrost rasti in metastatski potencial
  - Neimunogene



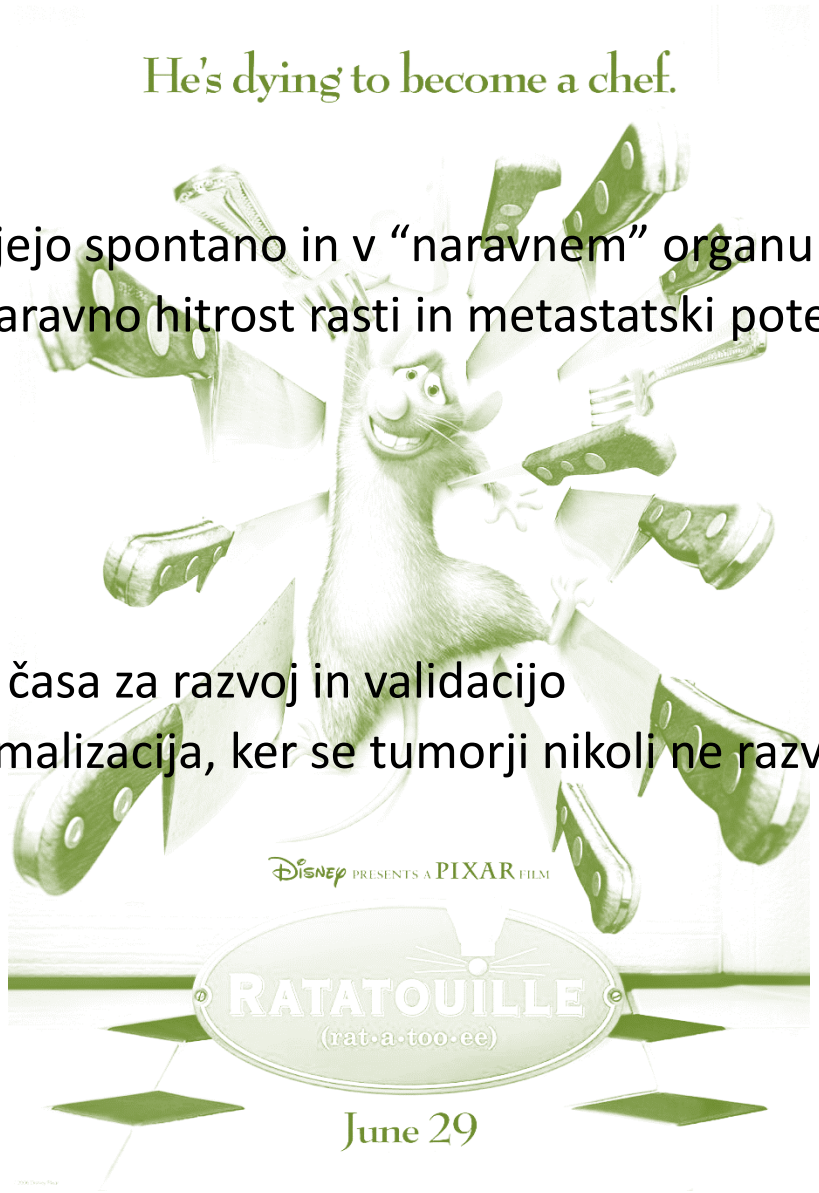
GREEN MICE – expressing green fluorescence protein

- Slabosti
  - Zelo drage
  - potrebno veliko časa za razvoj in validacijo

Knockout miši – delecija dol. gena

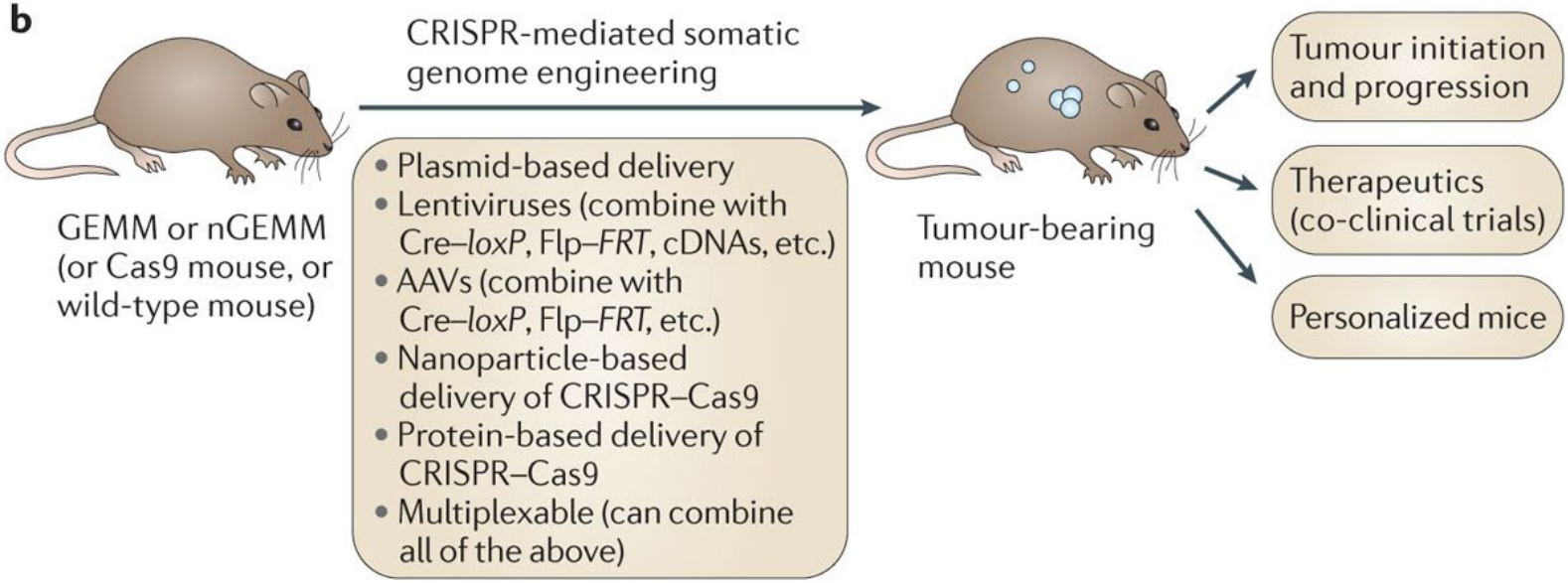
- Prednosti
  - Tumorji se razvijejo spontano in v “naravnem” organu
  - Tumorji imajo naravno hitrost rasti in metastatski potencial
  - Neimunogene

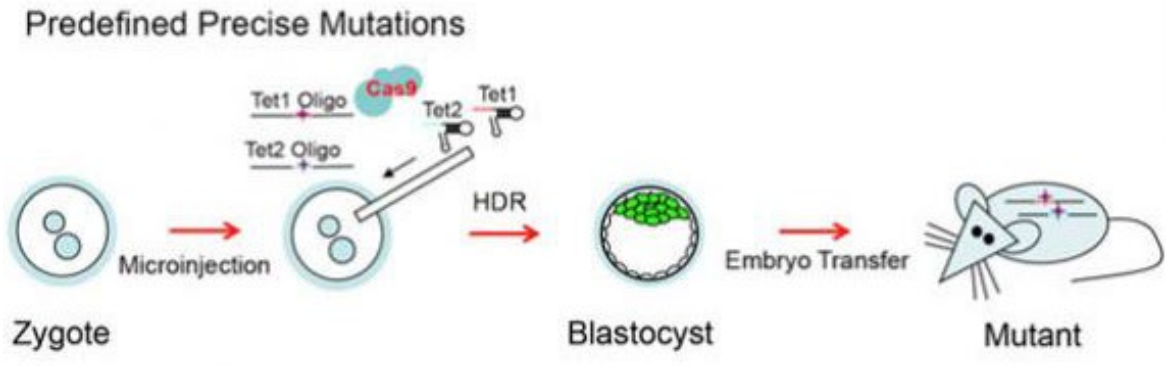
- Slabosti
  - Zelo drage
  - potrebno veliko časa za razvoj in validacijo
  - Potrebna je normalizacija, ker se tumorji nikoli ne razvijejo pri vseh živalih



Usmerjene mutacije

- Prednosti
  - Hitra producija
  - Relativno poceni





Humanizirane miši

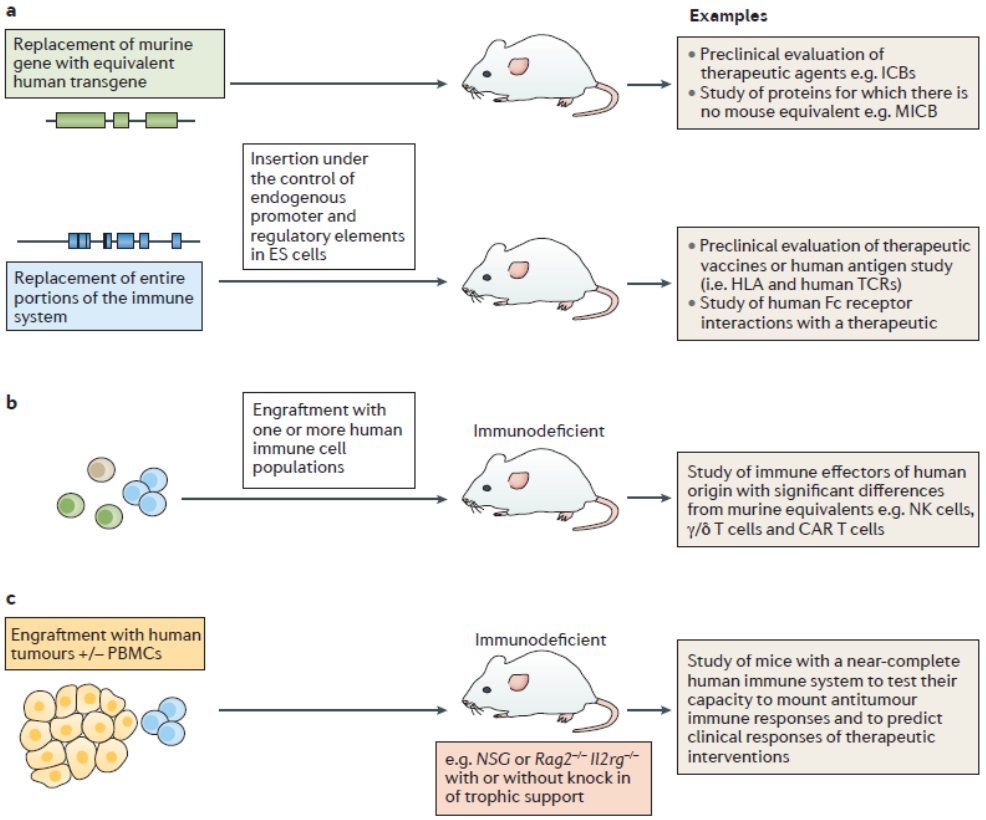
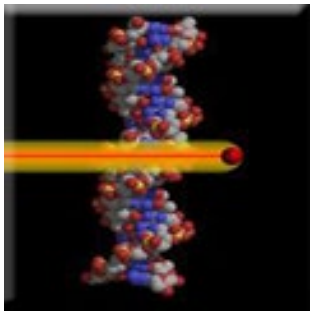


Figure 4 | **Humanized mouse models. a** | Genetic humanization of individual targets or of entire portions of the immune system. To overcome the considerable differences between some of the biological machineries in mice and humans, knock-in mice can be created by replacing a mouse immune gene with the human equivalent. Moreover, mouse immune genetic portions can be deleted and human equivalents (often encoded as transgenes) may be inserted into the mouse genome, resulting in the recapitulation of the unique profile of that human immune portion. **b** | Humanization of individual immune effectors. The specificity and function of several human immune effectors is different from that of rodents (for example, natural killer (NK) cells, as well as certain subsets of γ/δ T cells responding to lipid antigens). Immunodeficient mice (for example, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ and NSG mice) may be engrafted with such human immune cell populations to study their effects in human cancer models. **c** | Humanization for xenografts. Only mice bearing a major immunodeficiency can tolerate transplantation with human cells (for example, NSG mice). These mouse strains can be further optimized to support human innate immune effectors through the replacement of endogenous mouse cytokines with the human equivalents (for example, interleukin-3 (IL-3), granulocyte–macrophage colony-stimulating factor (GM-CSF) and thrombopoietin). Immunodeficient mice may be reconstituted with peripheral blood mononuclear cells (PBMCs) and tumours, ideally from the same patient, to predict clinical responses to novel therapeutic interventions. CAR, chimeric antigen receptor; ES, embryonic stem; HLA, human leukocyte antigen; ICB, immune checkpoint blocker; MICB, MHC class I polypeptide-related sequence B; TCR, T cell receptor.

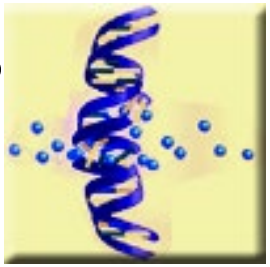
Zitvogel et al, Nature Reviews Cancer 2016; 16:759-773

Živalski modeli v radiobiologiji



- Planiranje radioterapije z drugimi načini zdravljenja (kemoterapijo, imunoterapijo gensko terapijo)
- Mehanizmi delovanja
- Reakcije normalnih tkiv (pozne reakcije pri kombiniranih zdravljenjih)

Živalski modeli v kemoterapiji oz. sistemskem zdravljenju



- Toksičnost
- Metabolizem zdravila
- Distribucija v telesu
- Tumorsko mikrookolje
- Učinkovitost
- Ne-klinično terstiranje pred začetkom klinični študije faze I

Mišji tumorski modeli

- Dostopnost in-breed linij (sorodniki)
- Tumorski sistemi lahko dostopni
- Relativno poceni
- Dobro definirani end-point-i
- Hitra rast tumorjev
- Hitri in ponovljivi rezultati

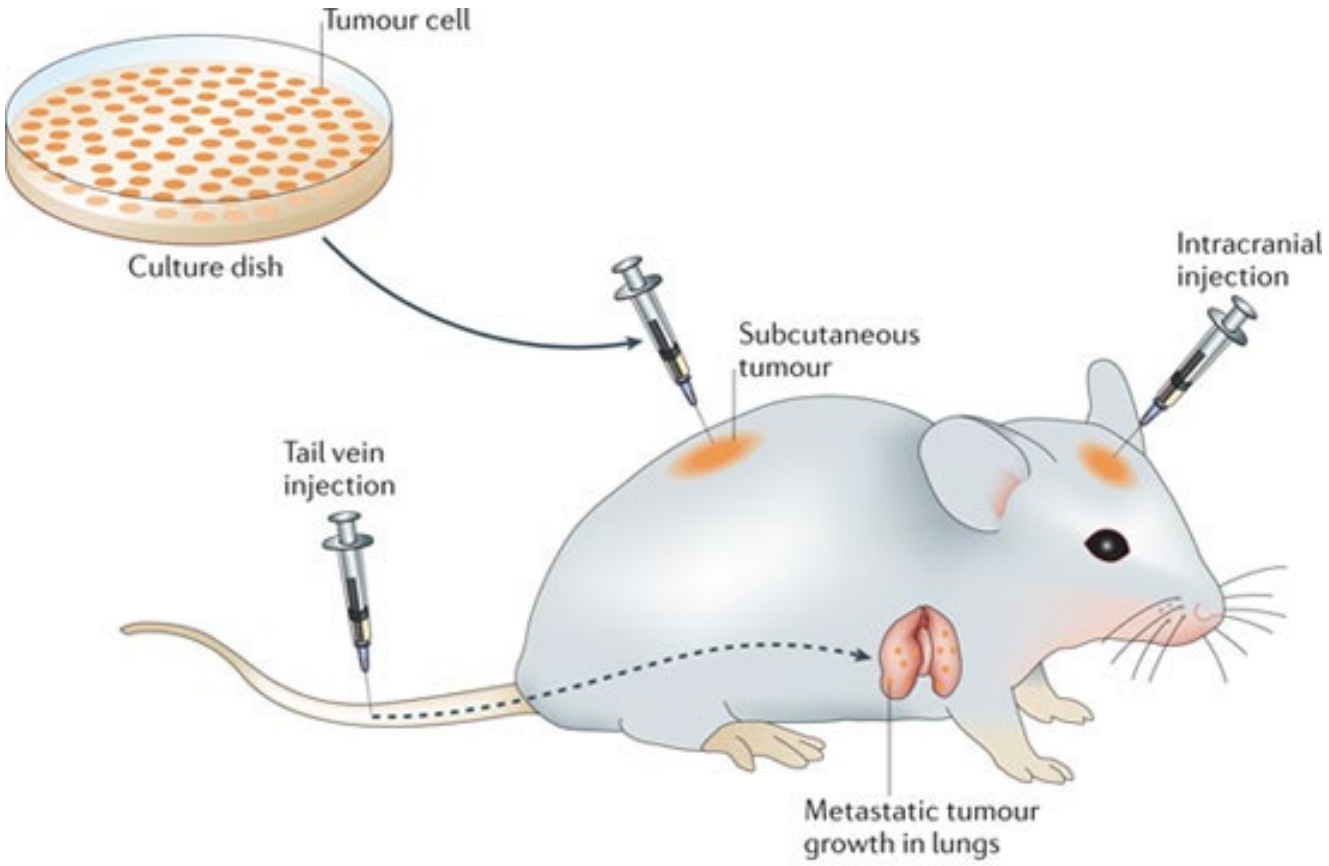
Spontani živalski tumorji

- Prednosti
  - Spontani v določenem sevu živali
  - Različni histološki tipi
  - Zelo podobni humanim tumorjem glede fizioloških parametrov in biologije rasti tumorjev
- Slabosti
  - Majhno število
  - Problematično načrtovanje poskusov
  - Slaba histološka karakterizacija
  - Majhen izbor primernih testov

Ustanovljeni presajeni tumorji – tumorji iz celičnih linij

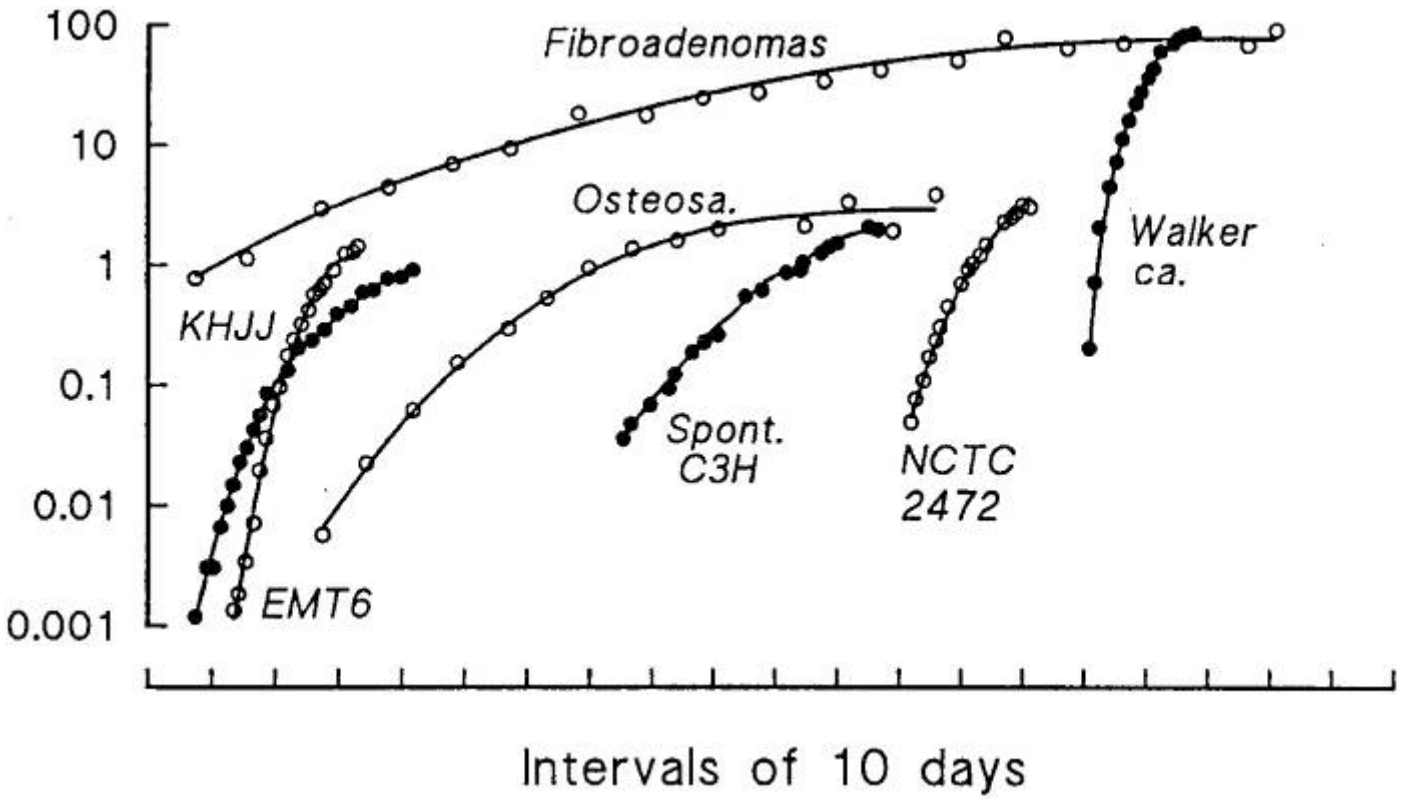
- Prednosti
  - Dobro histološko karakterizirani
  - Velik izbor primernih testov
  - Ponovljivost
- Slabosti
  - Velika celična selekcija
  - Običajno anaplastični

Presajeni tumorski modeli

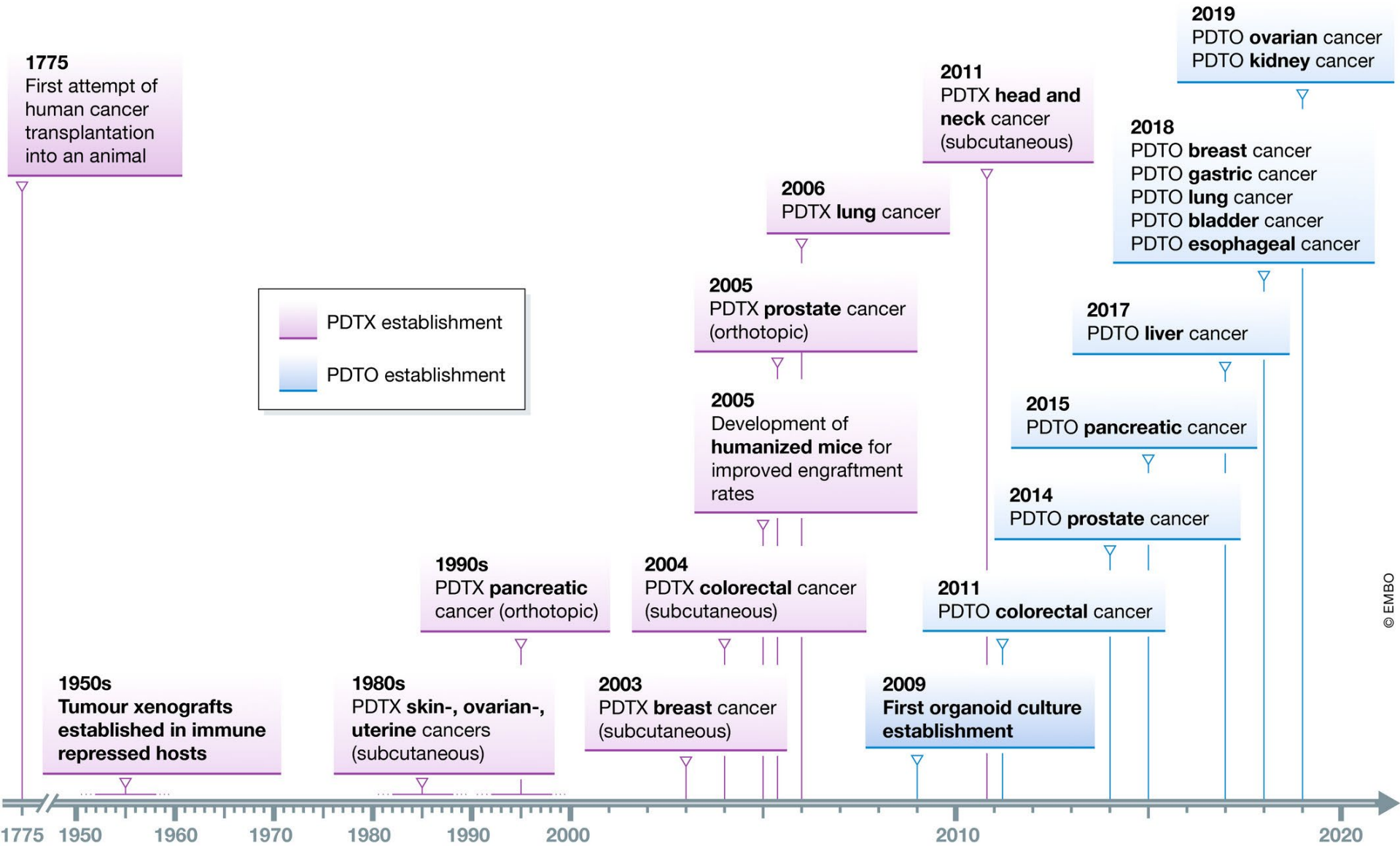


Cellular interactions
Immune responses

Rast tumorjev pri glodalcih



Ksenografti tumorjev bolnikov in organoidi tumorjev bolnikov

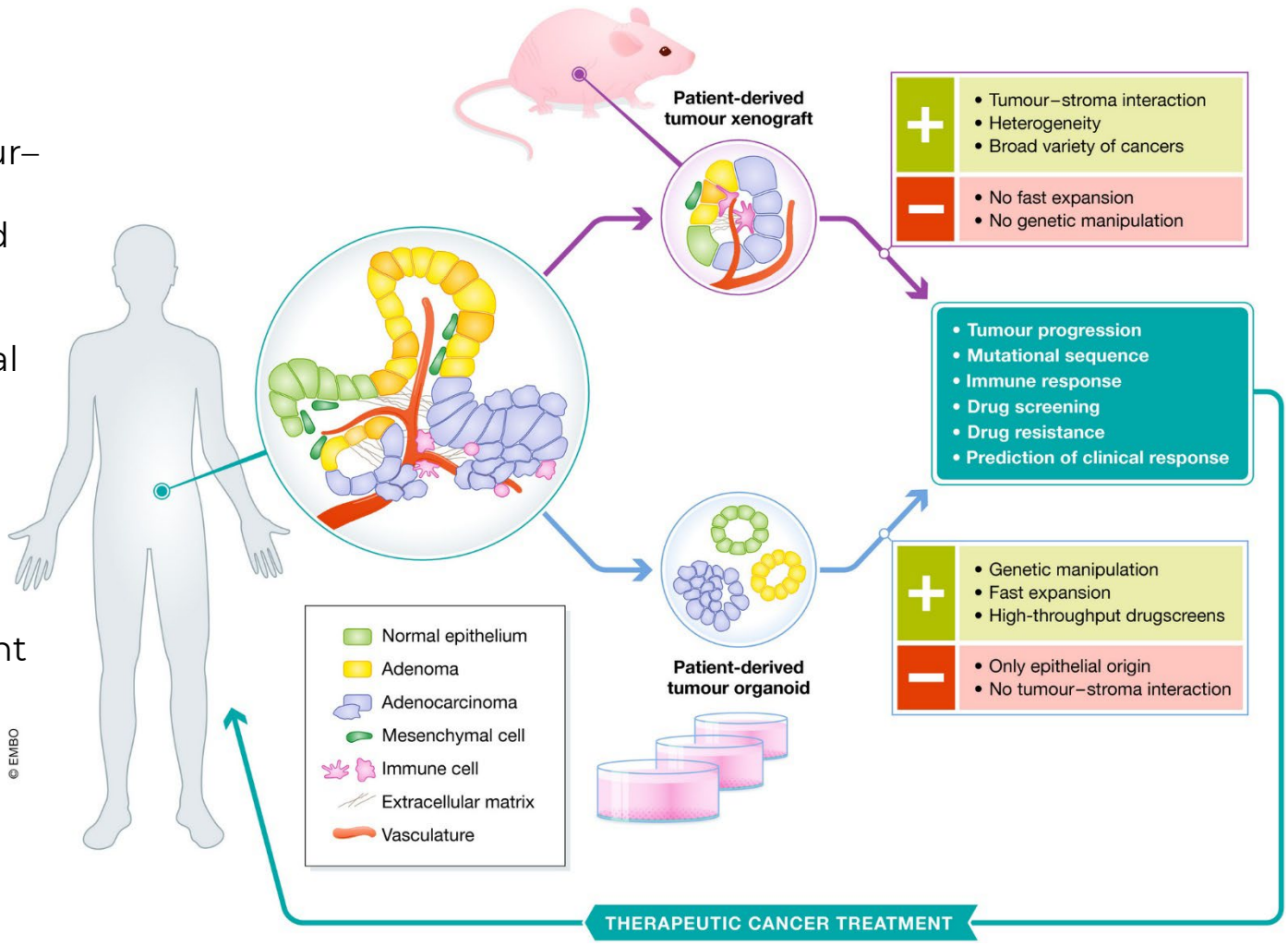


The EMBO Journal (2019)38:e101654

Patient-derived xenografts and organoides– ksenografti in organoidi tumorjev bolnikov

PDTXs preserve tumour heterogeneity and tumour–stroma interactions. PDTOs grow in a provided basement membrane extract and can be established from epithelial cancer cells as well as normal epithelial tissue.

Both models allow for several translational applications that contribute to development of therapeutic cancer treatments. Part of this figure was adapted from Sachs and Clevers (**2014**).



EMBO J, Volume: 38, Issue: 15, First published: 08 July 2019, DOI: (10.15252/embj.2019101654)

Humani tumorski ksenografti

- Prednosti

  Morfološke in biološke karakteristike originalnih tumorjev so ohranjene

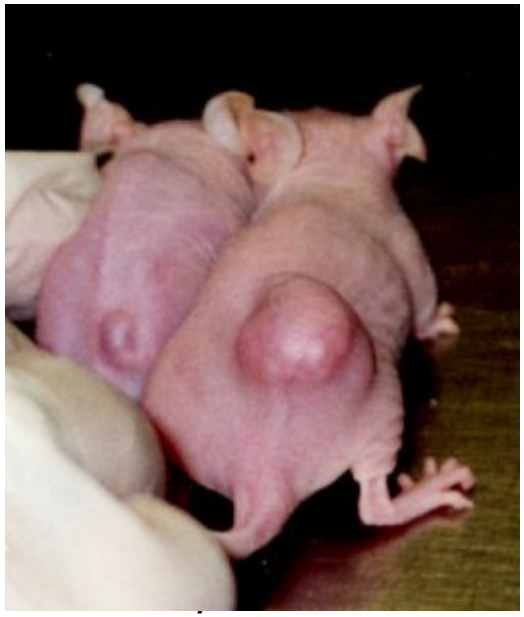
  Dobro odražajo odgovor na zdravljenje

- Slabosti

  Vaskularizacija je specifična za gostitelja, ne za tumor

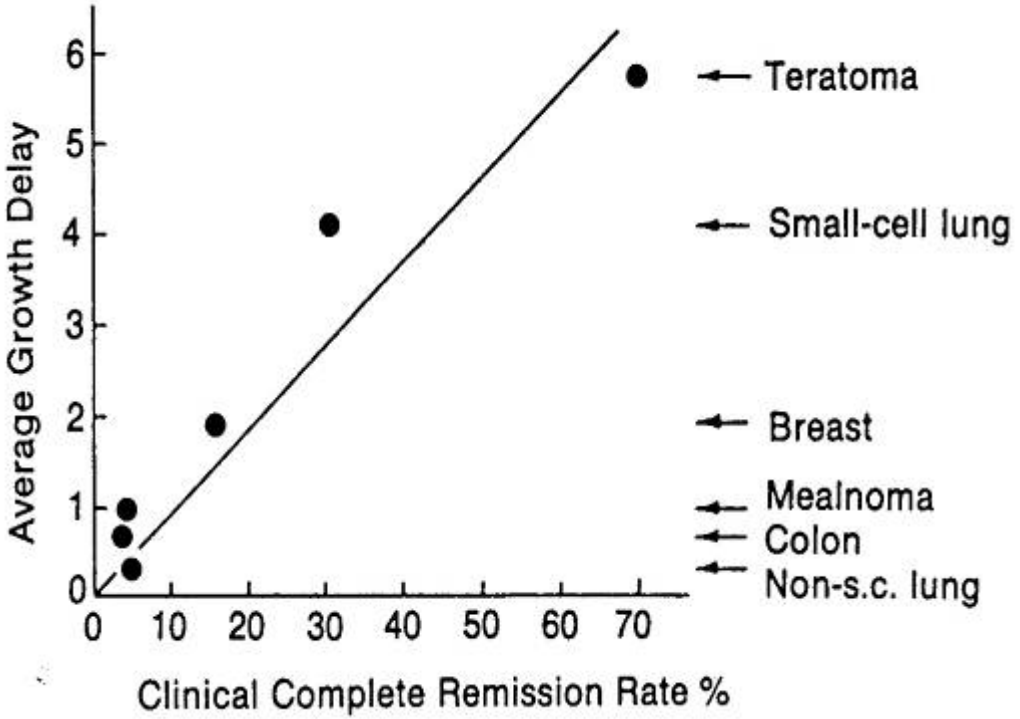
  Podvojitveni čas rasti tumorja je krajši

  Imunski odziv organizma

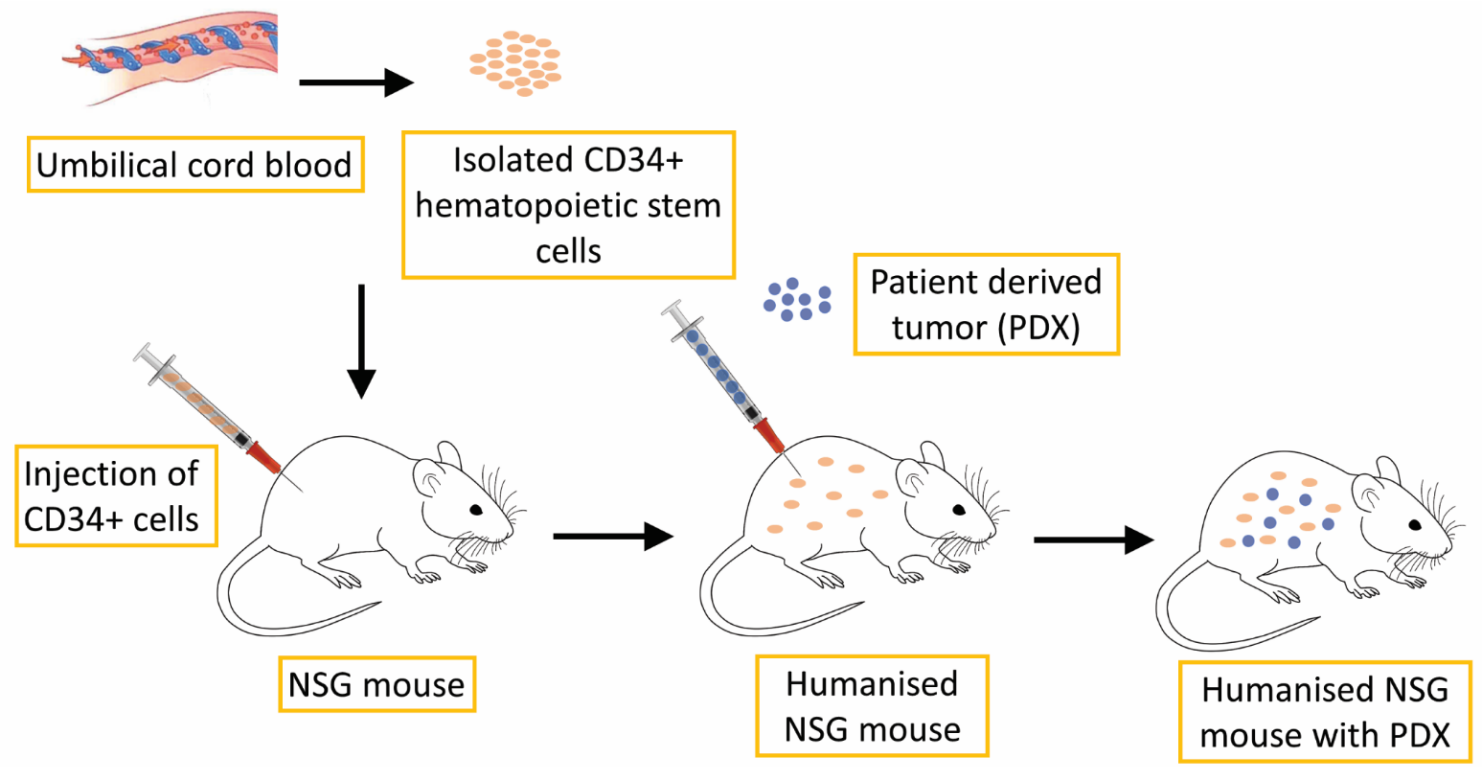


Humani tumorski ksenografti

- Korelacija med odgovorom med ksenografti in kliničnim odgovorom pri pacientih po kemoterapiji

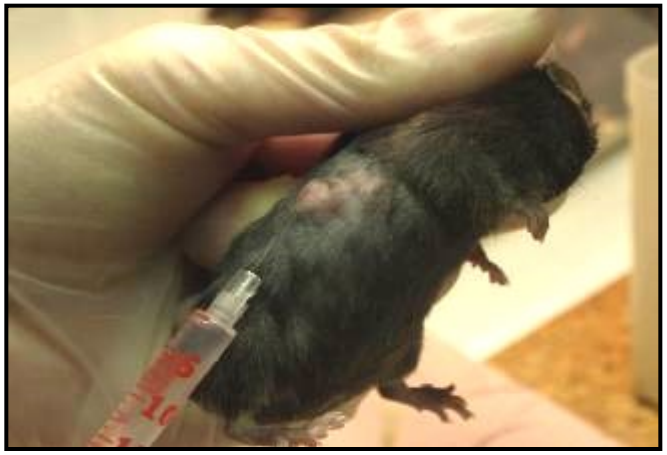


Humanizirane miši in Patient-derived xenograft

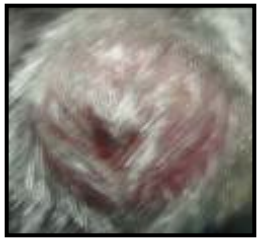


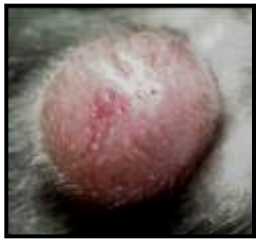
Lampreht Tratar, Horvat, Čemažar, Frontiers in Oncology 2018 doi: 10.3389/fonc.2018.00266

Podkožni tumorji, pljučne metastaze



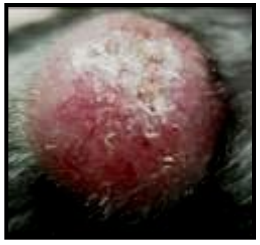
Kontrola





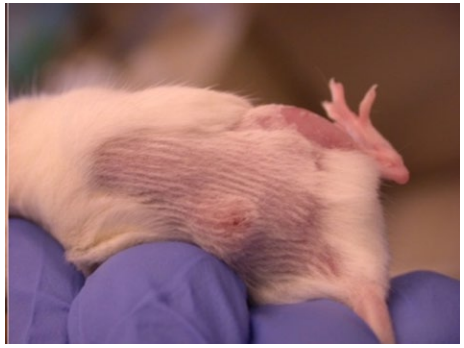
Bleomicin
20 μg/miš

EP: 8 pulzov,
1300 V/cm
1 Hz, 100 μs

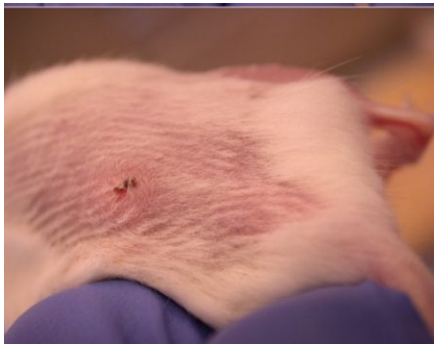


Elektrokemo terapija

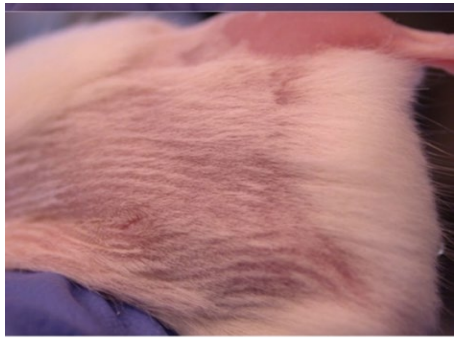
Protitumorska učinkovitost



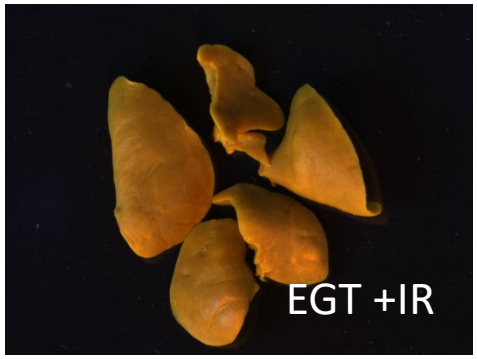
Day 0



Day 7



Day 14



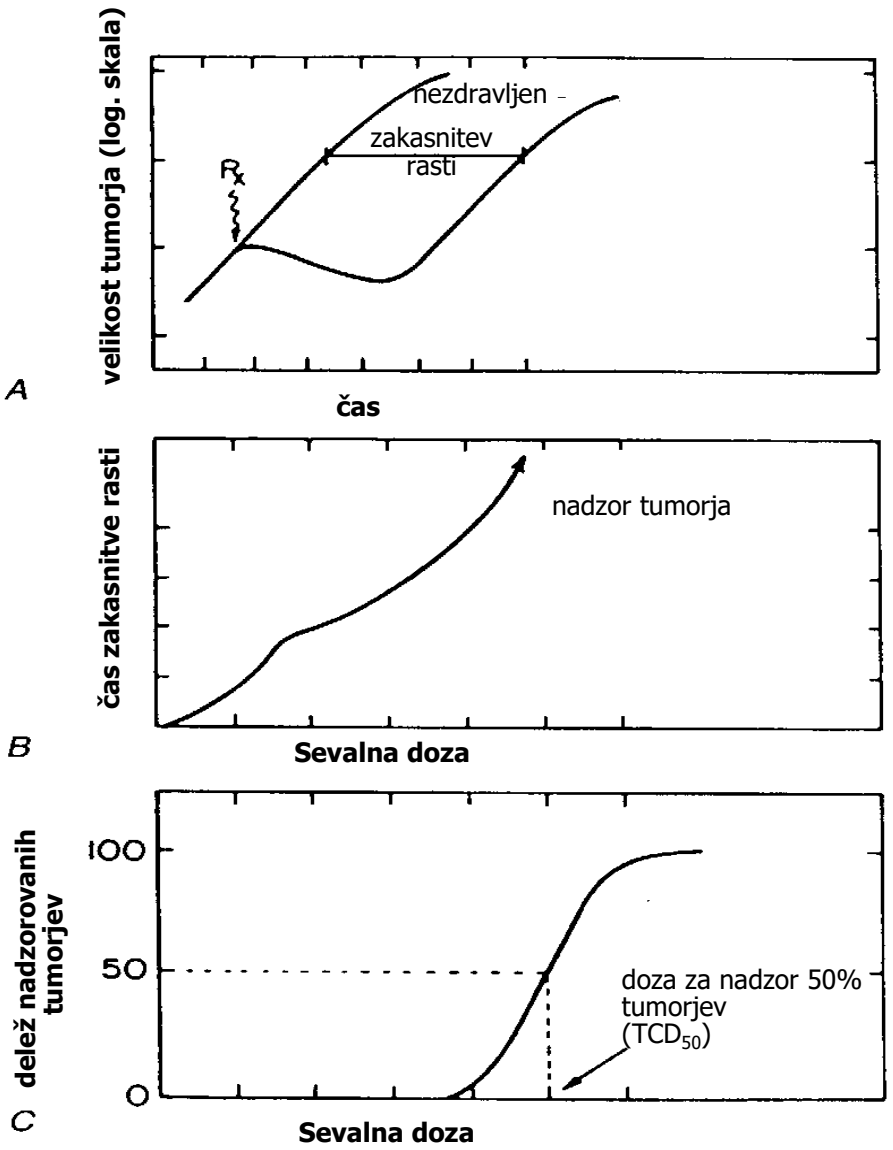
Učinki zdravljenja – stopnja kontroliranja bolezni v humani onkologiji – korelacija z predkliničnimi modeli

- RECIST (Response evaluation Criteria in Solid Tumors) kriteriji:
  - Popolni odgovor: popolno uničenje tumorja
  - Delni odgovor: tumor zmanjšan za več kot 50%
  - Stagnacija (stable disease): zmanjšanje za manj kot 50%
  - Napredovanje bolezni: rast tumorja

- Trajanje uspeha: 5-letno preživetje - ~ 100 dni pri miših
  - Lokalno stanje bolezni
  - Preživetje – celokupno preživetje; preživetje brez ponovitve bolezni, preživetje do pojava simptomov…

Testi za ugotavljanje protitumorske učinkovitosti

- Test zaostanka v rasti tumorjev
- Test lokalne kontrole rasti tumorjev



Krivulje preživetja – trajanje uspeha

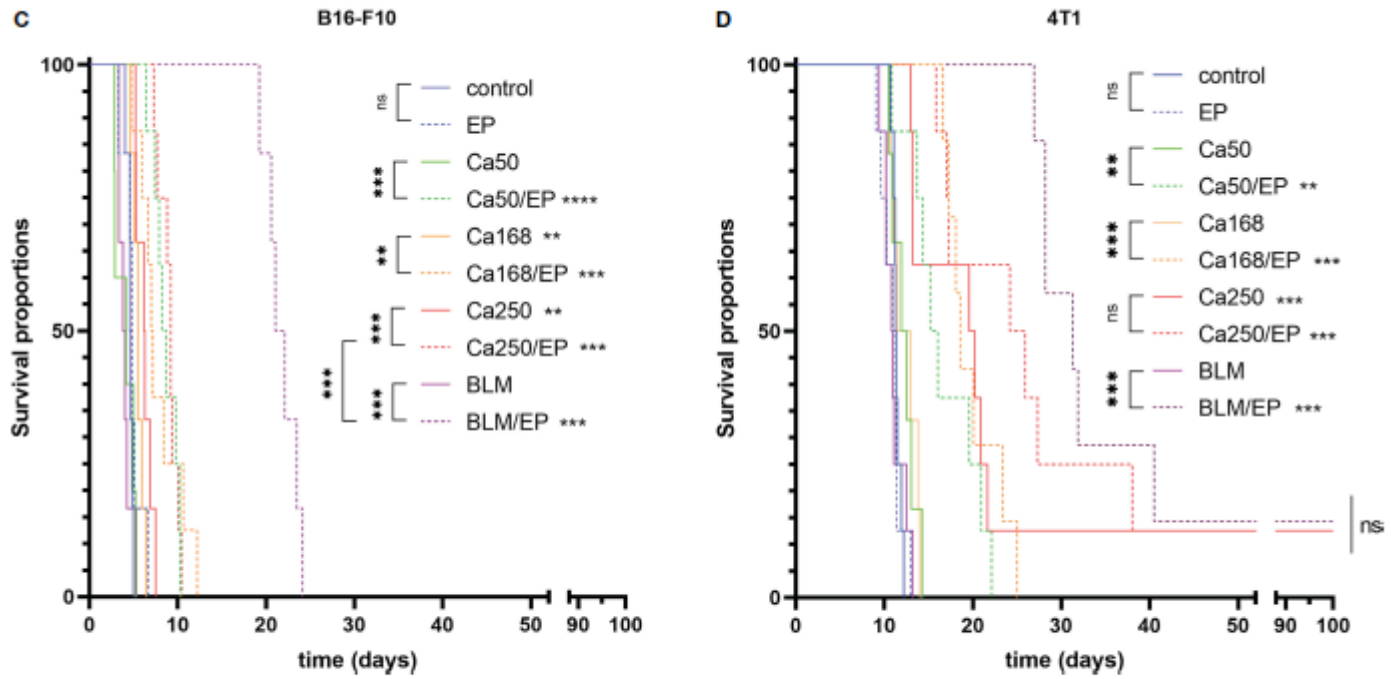
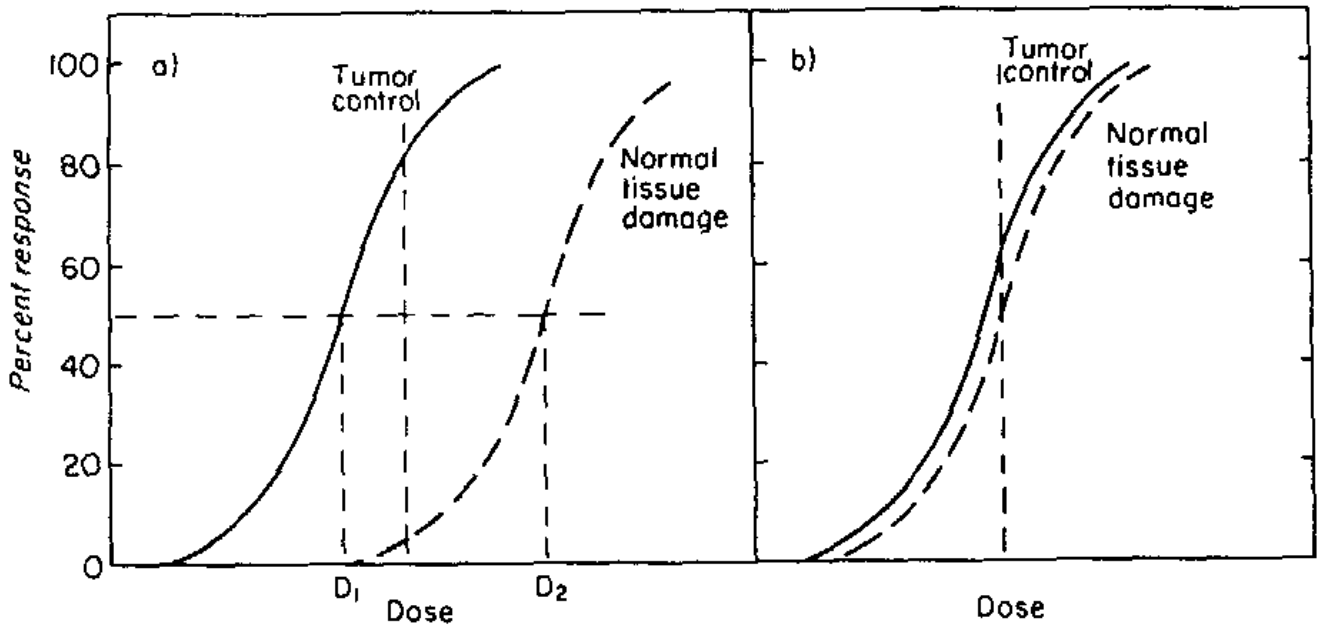


FIGURE 3
Effectiveness of CaEP or ECT with bleomycin is different in B16-F10 and 4T1 tumors. The response to the therapy is presented by **(A, B)** tumor growth delay and **(C, D)** Kaplan-Meier graphs. In **(A, B)** each dot represents one animal in violin plots showing distribution of data. ns $P \geq 0.05$; *P 0.01 – 0.05; **P 0.001 – 0.01; ***P 0.0001 – 0.001; ****P <0.0001.

Poškodbe normalnih tkiv

- Testi za določevanje poškodb
  - *Spošno stanje tkiv (koža)*
  - *Funkcionalni testi za določene organe:*
    - Hitrost dihanje (pljuča)
    - EDTA izločanje (ledvica)
    - Krvni testi (kostni mozeg)
  - *Testi klonogenosti (in situ ali presajeni na drugo lokacijo)*

Terapevtski indeks



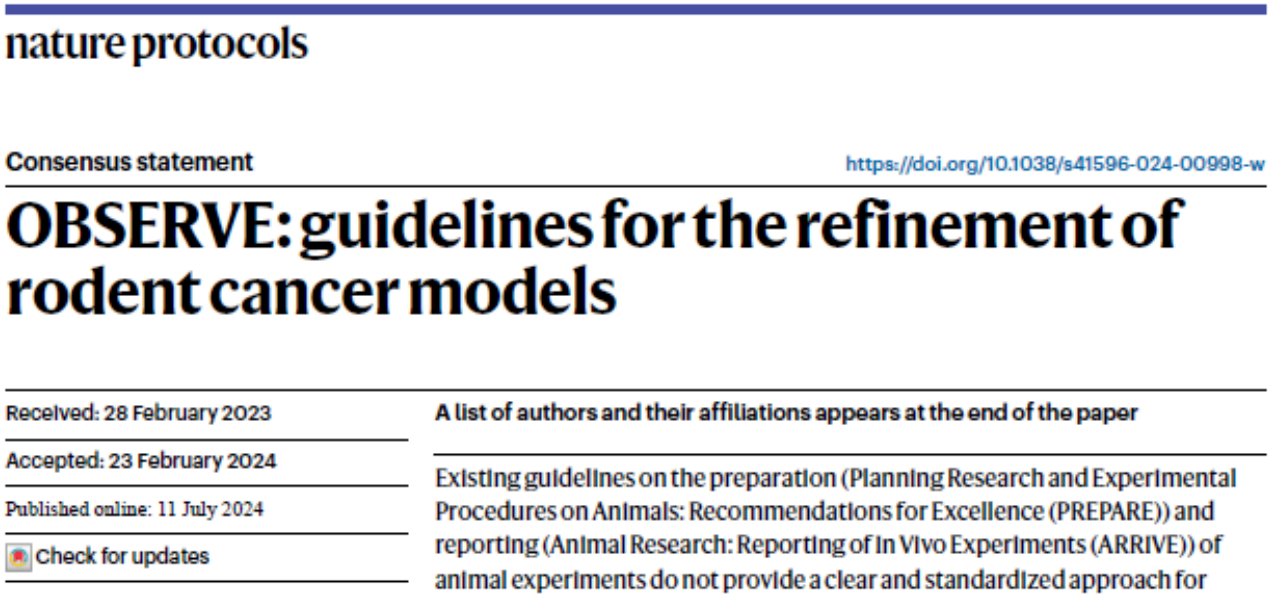
Terapevtski indeks določa odgovor tumorja pri točno določeni poškodbi normalnega tkiva.

Smernice za dobrobit živali s tumorji - splošno

- 3 R’s (reduction, refinement, replacement)
- Poudarek na napovedovanju in prepoznavanju stranskih pojavov in uporabi humanih “end-pointov”
- Načrtovanje poskusov (pilotne študije, statistika, učenje, sistem dokumentacije)

OBSERVE smernice

- Sistematično spremljanje živali za zagotovitev dobrega počutja in kakovostnih rezultatov.



nature protocols

Consensus statement

https://doi.org/10.1038/s41596-024-00998-w

OBSERVE: guidelines for the refinement of rodent cancer models

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Check for updates

A list of authors and their affiliations appears at the end of the paper

Existing guidelines on the preparation (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE)) and reporting (Animal Research: Reporting of In Vivo Experiments (ARRIVE)) of animal experiments do not provide a clear and standardized approach for

Smernice za dobrobit živali s tumorji - specifično

- Določevanje resnosti poskodb
- Biologija tumorjev
- Natančno načrtovanje poskusov (humano)
- Spremljanje živali
- Dokumentacija in objave

https://doi.org/10.1038/s41596-024-00998-w

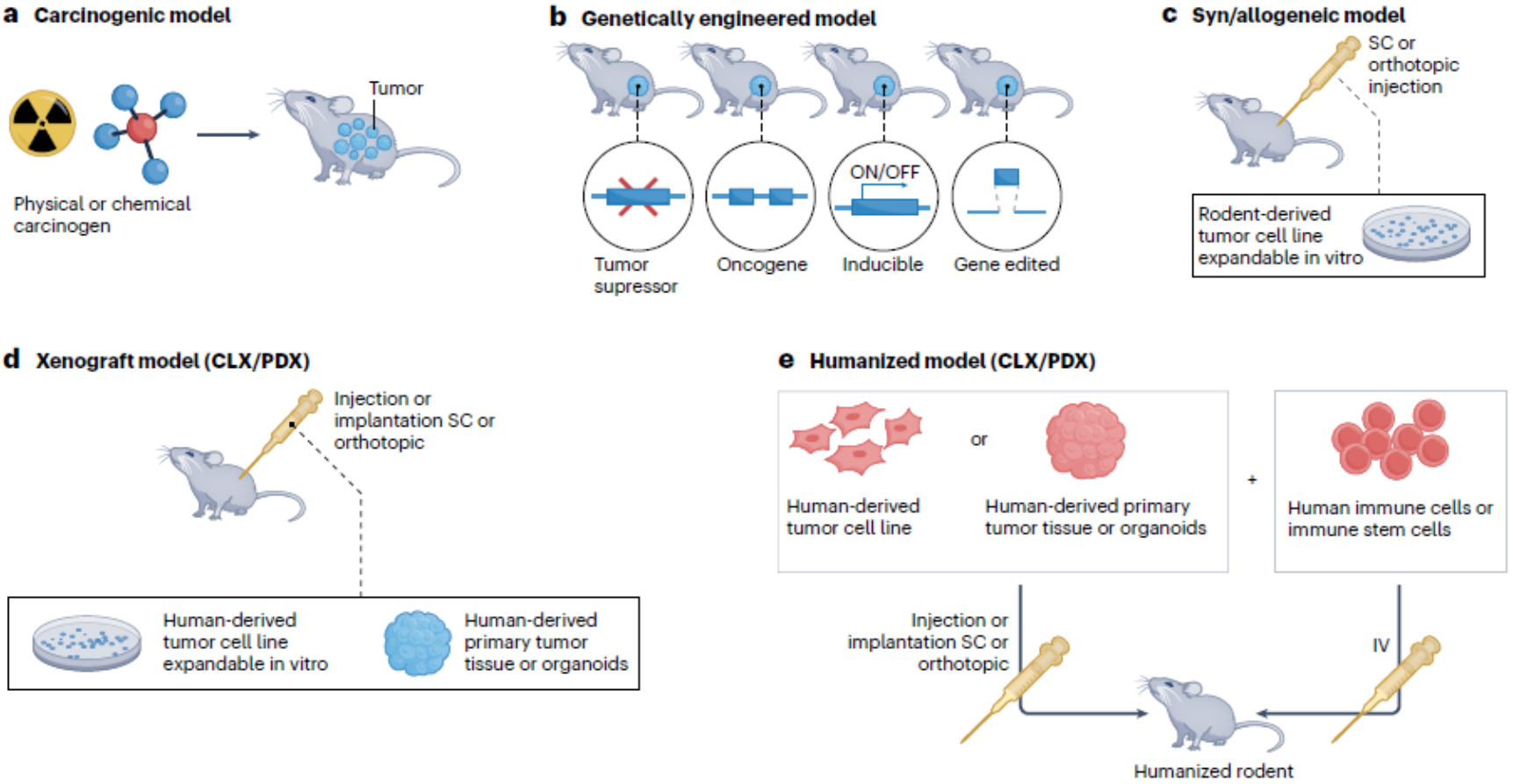


Fig. 1 | Schematic overview of types of rodent tumor model. a, A carcinogenic model: cancer model established in immunocompetent animals by applying a physical and/or chemical carcinogen(s) inducing genetic alterations causing tumor growth. **b**, A genetically engineered model: cancer model based on the genetic alteration of oncogenes or tumor suppressor genes or administration of exogenous activating agents for organ-specific tumor development. **c**, A syngeneic model and allogeneic model: engraftment of mouse or rat tumor cells or organoids in, respectively, a genetically identical, immune competent strain or a genetically different, immune deficient strain of the same species. Cell lines can be generated from a chemically induced or genetically engineered model. **d**, A xenograft model: xenograft in immune deficient animals of a cell line, a tissue piece or organoids generated from a cancer biopsy, usually from human origin. **e**, A humanized model: xenograft model in immunodeficient mice previously engrafted with human immune cells. CLX, cell line xenograft; PDX, patient-derived xenograft.

BOX 1

Tumor measurement with a caliper

The length of the tumor is intended as its longest axis; the width as its shortest axis; and the depth is usually not used in the calculation (because it is hard to measure accurately), instead it is preferred to use the smallest measurement (i.e., width) twice. The following formulas may be used to measure tumor volume:

1. **Ellipsoid variants**
   $4/3\pi r_1 r_2^2$
   where r_1 is the longest radius and r_2 is the shortest radius assuming width and depth of the tumor are equal or,
   $4/3\pi r_1 r_2 r_3$
   where r_1 is the longest radius and r_2 and r_3 are the shortest radius (width and depth) or,
   $(\pi a b^2)/6$
   where *a* is the longest axis and *b* is the shortest axis, assuming width and depth of the tumor are equal or,
   $(\pi a b c)/6$
   where *a* is the longest axis, and *b* and *c* are the short axes.
2. **Modified ellipsoid**
   $(a b^2)/2$
   where *a* is the longest axis and *b* is the shortest axis, assuming width and depth of the tumor are equal.

Image **a** of the figure shows the axes and radiuses of a SC tumor, which are used to measure tumor volume; the correct placement of the caliper is shown in **b**; and an incorrect placement of the caliper is shown in **c**. Correct placement of the caliper is of extreme importance for the correct measurement of tumor volume. As a rule of thumb: it should always be possible to move the caliper over the tumor when measuring the length/height. As such, the tumor is not 'squeezed' and its volume not underestimated (notice the difference in volume measurement between **b** and **c**). To avoid observer bias, a blind study design should be set up especially in treatment studies.

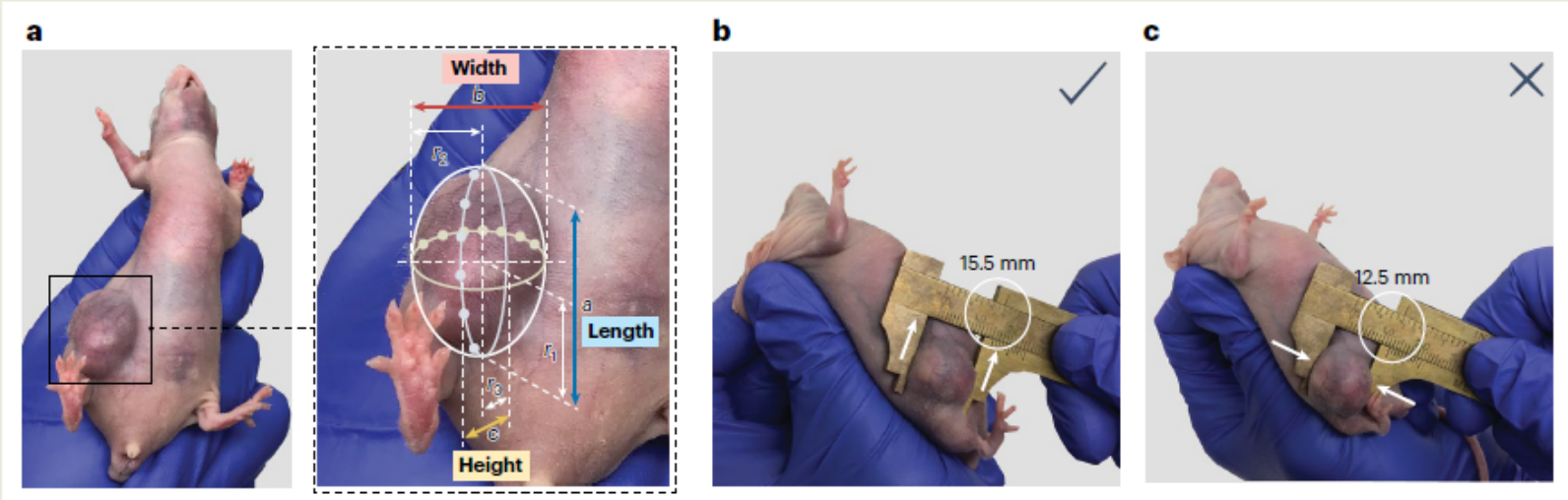


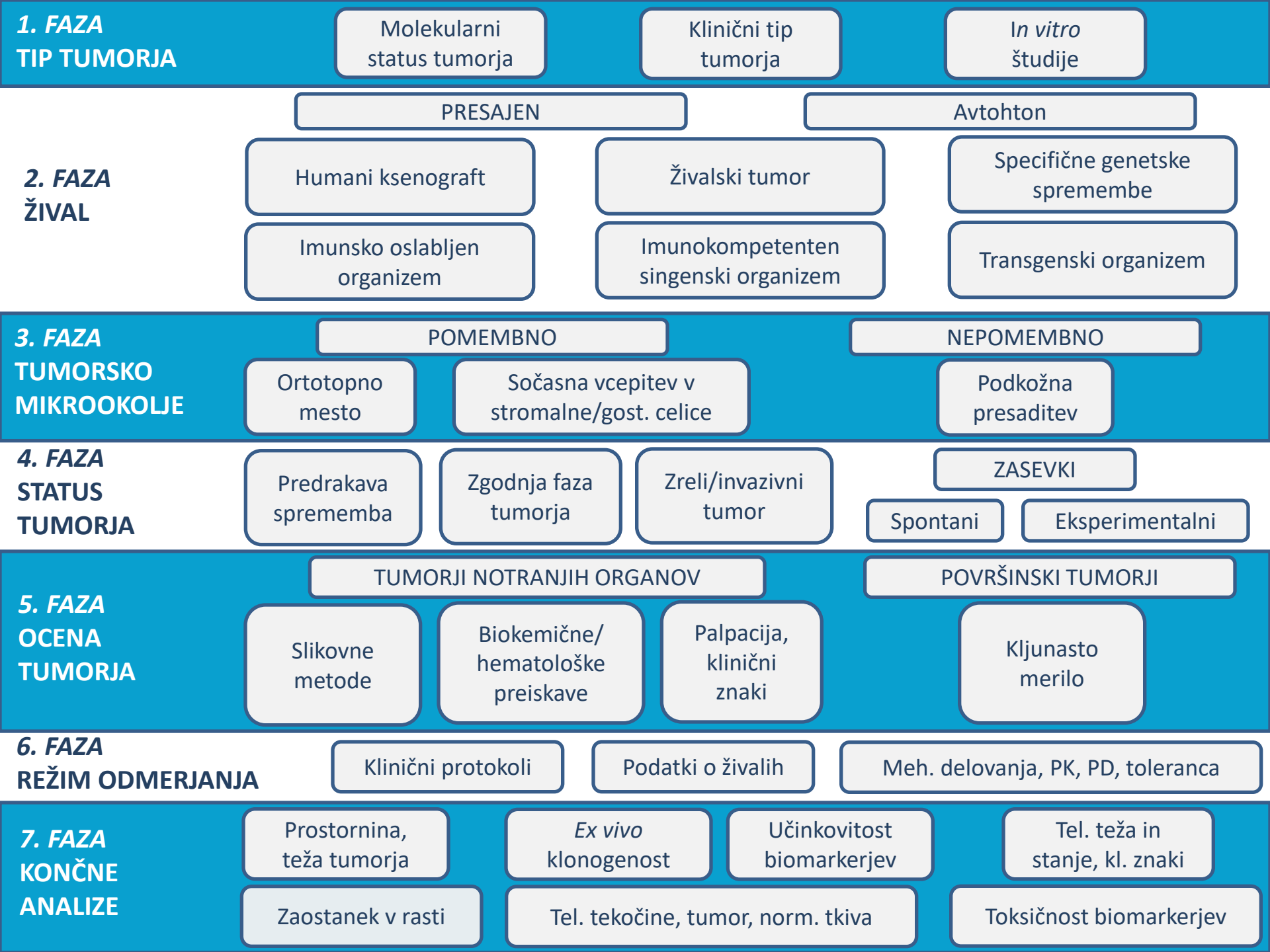
Table 1 | Clinical signs associated with organ-/system-specific tumor development

	General signs				Specific signs									Refs.
	Anorexia and BW loss[a]	Altered appearance and behavior	Pain: demonstrated	Pain: assumed	Icterus	Respiratory signs	Ascites	Anemia	GI signs	Neurological signs	Locomotor signs	Urinary signs	Cutaneous ulceration	
GI tract														
Oral cavity	x	x	x											128–131
Esophagus	x	x		x										132,133
Gastric	x	x		x	x		x	x	x					134–137
Pancreas	x	x	x		x		x							34,130, 131,138,139
Colorectal	x	x	x					x	x					33,140–142
Liver + gallbladder	x	x		x	x		x							143–146
Urogenital system														
Kidney (+ adrenal)	x	x		x								x		147
Bladder	x	x	x									x		148–152
Prostate	x	x		x								x		153–156
Uterus	x	x		x			x	x				x		157
Ovary	x	x		x			x					x		55,158,159
Cervix	x	x		x								X		160–162
Mammary	(x)	x	x								x		x	131
Nervous system														
Brain tumors	x	x		x						x				79,163,164
Other CNS	x		x							x	x			130,131,165
Peripheral nerve	x	x	x				x	x		x	x			166
Soft tissue and bone														
Soft tissue	Clinical signs of soft tissue tumors depend on the location, please refer to other organs to have an indication of adverse effects													
Bone (+ multiple myeloma bone disease)	x	x	x								x			130,131, 167,168

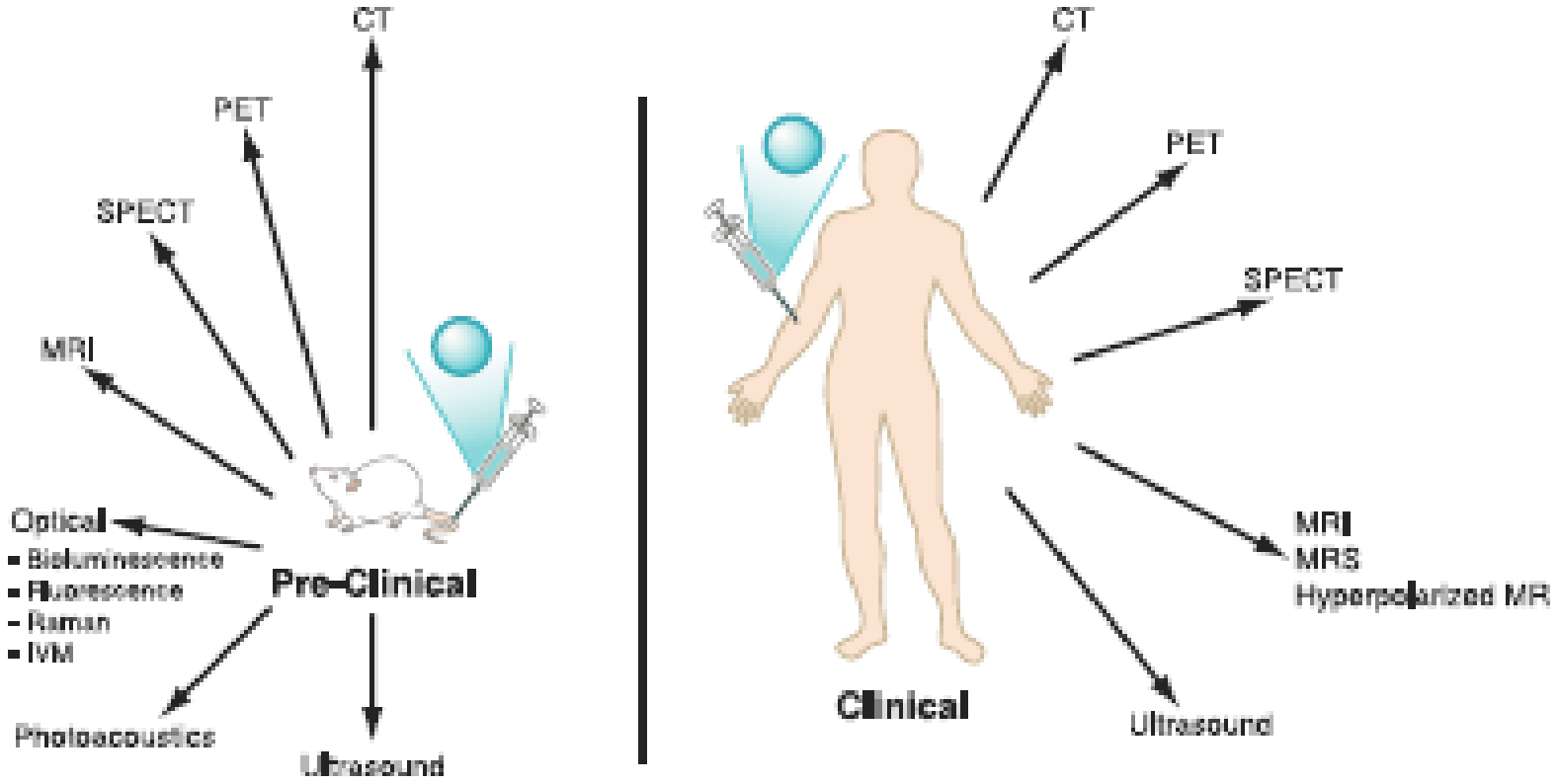
Table 2 | Clinical signs associated with cancer models: description, recognition and etiology

Clinical sign	Description	How to assess?	Etiology
General signs			
Anemia	Reduced number of red blood cells and/or low hemoglobin	Evaluate the color of the mucous membranes (around the eyes, mouth and tongue) and skin (interdigital spaces), which should be pink. If they are pale, anemia is probably present.	Blood loss in cancer is usually due to ulcerating tumors, necrosis or inflammation especially in GI or urogenital tumors. A decreased production of red blood cells may be present in tumors involving the bone marrow (e.g., leukemia, lymphoma or bone marrow metastasis), inflammation present in chronic conditions (such as cancer) or myelosuppressive (chemo/radio)therapy. Anemia may also be seen in GVHD as excessive immune activation leads to hemolysis of erythrocytes by foreign antibodies.
Anorexia	Loss of appetite	Is usually seen as weight loss. Weight loss can be determined by weighing the animals (see Box 4 for more information on weight during tumor studies). In many cancer models, body condition score (BCS) is the preferred monitoring tool[182,183]. The absence or marked reduction of fecal pellets in the cage may also be an indication of anorexia. Food intake can be assessed in a metabolic cage (requires single housing) or by weighing food. In cancer studies this is often not done or even necessary.	Anorexia, accompanied by BW loss and ultimately dehydration (see below), can be an adverse effect of many tumor types but is also a known side-effect of commonly used (chemo) therapeutics such as cisplatin. Weight loss can also result from the release of biologically active substances by the tumor or the host.
Ascites	Fluid accumulation in the abdomen	Seen as abdominal distention that may quickly increase; animal looks potbellied. The distension can usually be depressed in contrast to abdominal distension resulting from a mass or organ enlargement.	Cancer can metastasize to the peritoneum leading to vascular and lymphatic permeability, and subsequently, the accumulation of fluid causing malignant ascites. Tumors can also metastasize to the liver or lymphatic system causing increased pressure, hampering the return of fluid from the abdomen to the heart.
Cachexia	A 'wasting' disorder that results in extreme weight loss, loss of muscle and adipose tissue	Loss of muscle and fat results in skeletal structures being clearly visible, animals have a BCS[182,183] of 1.	Cachexia is a common complication in cancer. It is caused by catabolism factors produced by tumors and physiological factors such as proteolysis and autophagy. Cachexia is characterized by anorexia and metabolic disturbances
Dehydration	Reduction in total body water	Dehydration can be assessed by pinching and gently lifting (tenting) the skin over the shoulder blades (skin turgor test). Normally, the skin will quickly return to its original shape. If the tent remains or is slow to resolve, the animal is dehydrated. In the case of severe dehydration, animals may be weak, immobile and may have sunken eyes[184].	Caused by an imbalance between uptake and loss of fluids. In cancer, this is usually due to anorexia (see above) but can also be a consequence of severe diarrhea (see below).
Icterus	Yellow discoloration of the skin, mucus membranes and eyes, also known as jaundice or hyperbilirubinemia	Yellow pigmentation of the skin, tissues and body fluids; usually observable in the hairless parts of the body. Icterus is easier to see in hairless or albino mice.	Icterus is caused by the accumulation of bilirubin that cannot be metabolized by the liver and secreted in the bile due to cancer of the liver, chemotherapy or obstruction of the bile duct by a tumor.

Izbira in uporaba tumorskega modela


1. FAZA
TIP TUMORJA
Molekularni status tumorja
Klinični tip tumorja
In vitro študije
PRESAJEN
Avtohton
2. FAZA
ŽIVAL
Humani ksenograft
Živalski tumor
Specifične genetske spremembe
Imunsko oslabljen organizem
Imunokompetenten singenski organizem
Transgenski organizem
3. FAZA
TUMORSKO MIKROOKOLJE
POMEMBNO
NEPOMEMBNO
Ortotopno mesto
Sočasna vcepitev v stromalne/gost. celice
Podkožna presaditev
4. FAZA
STATUS TUMORJA
Predrakava sprememba
Zgodnja faza tumorja
Zreli/invazivni tumor
ZASEVKI
Spontani
Eksperimentalni
5. FAZA
OCENA TUMORJA
TUMORJI NOTRANJIH ORGANOV
POVRŠINSKI TUMORJI
Slikovne metode
Biokemične/ hematološke preiskave
Palpacija, klinični znaki
Kljunasto merilo
6. FAZA
REŽIM ODMERJANJA
Klinični protokoli
Podatki o živalih
Meh. delovanja, PK, PD, toleranca
7. FAZA
KONČNE ANALIZE
Prostornina, teža tumorja
Ex vivo klonogenost
Učinkovitost biomarkerjev
Tel. teža in stanje, kl. znaki
Zaostanek v rasti
Tel. tekočine, tumor, norm. tkiva
Toksičnost biomarkerjev

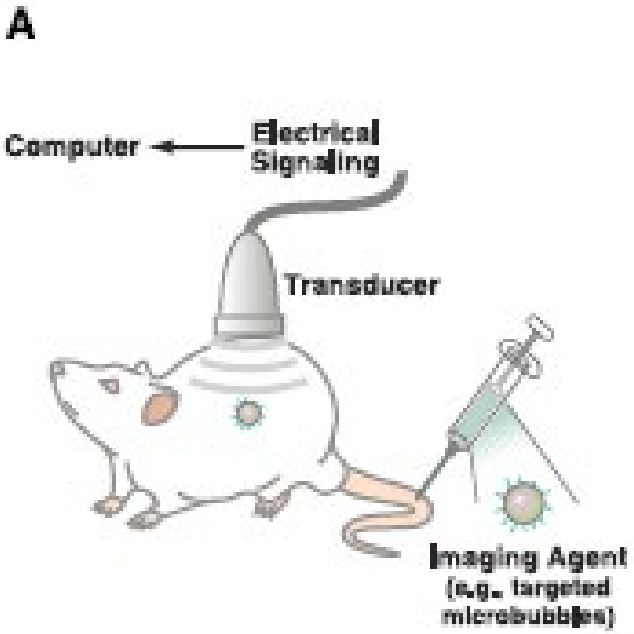
Molecular imaging modalities used for preclinical and/or clinical applications

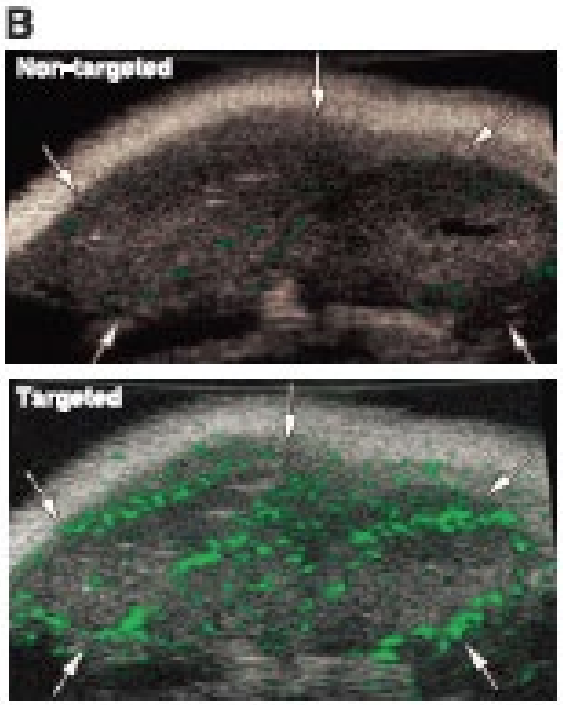


Molecular imaging – main field of study

- Monitoring deep seated tumors with or without therapy
- Study of basic biological processes
- Tissue pharmacokinetics and pharmacodynamic responses to treatment
- Pharmacodynamic imaging of molecular targeted therapeutics

Small animal ultrasound - US





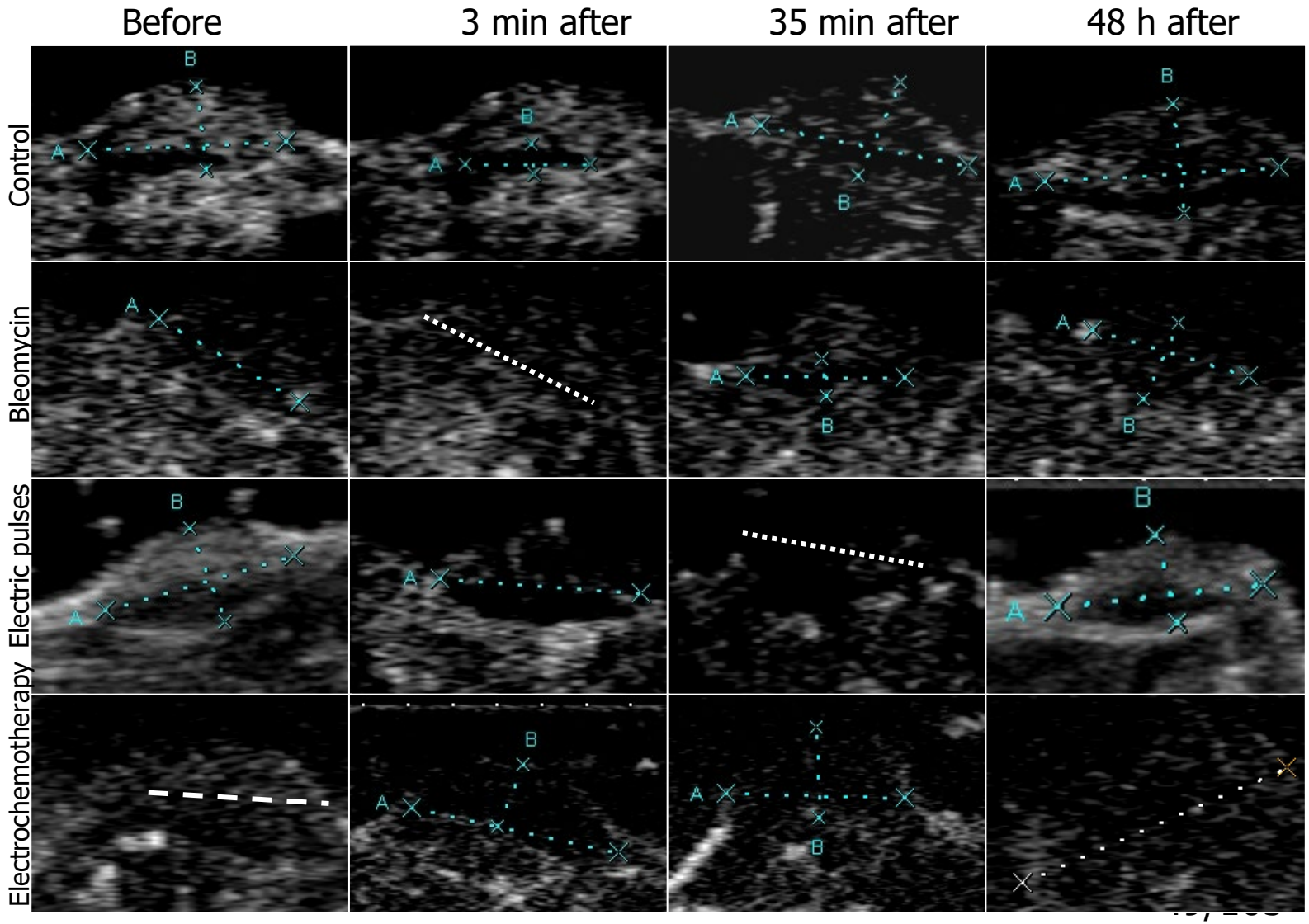
Advantages

- Relatively inexpensive
- No ionizing radiation
- Good temporal resolution
- Quantitative data
- Excellent sensitivity with microbubbles
- Clinical utility

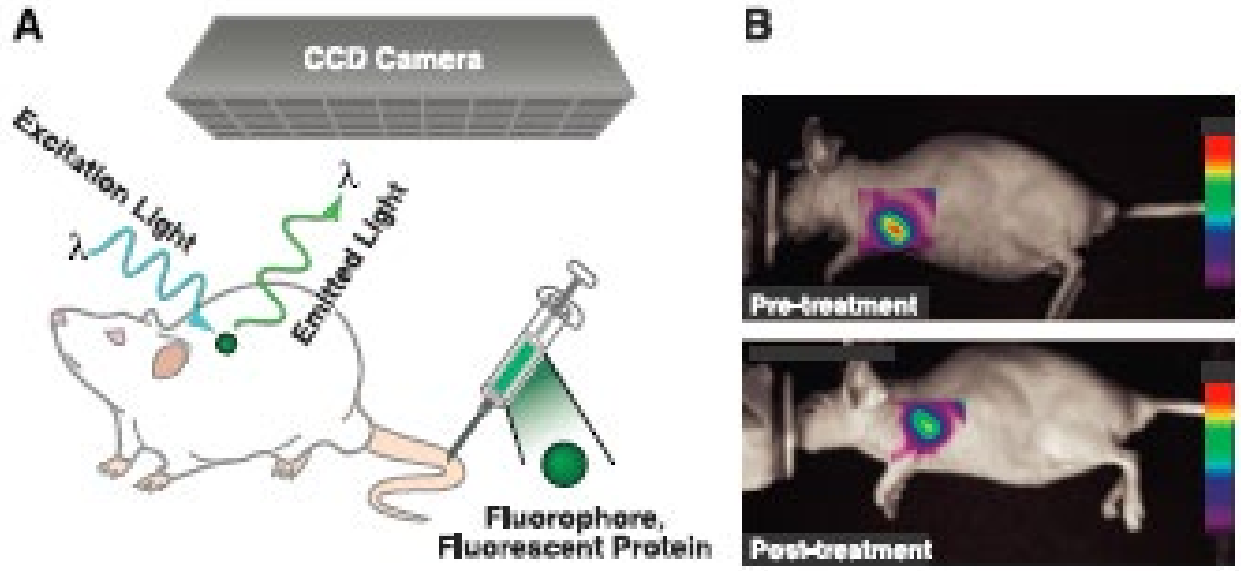
Drawbacks

- Limited depth of penetration
- Primarily anatomical information
- Limited molecular imaging applicatio
- Limited to imaging of soft-tissues onl

Perfusion of tumors after electrochemotherapy – US with contrast agent



Small animal optical fluorescence imaging



Advantages

- Relatively inexpensive
- User friendly
- Multiplexing capabilities

Drawbacks

- Limited depth of penetration
- Poor spatial resolution at greater depths
- Surface weighted images
- Autofluorescence

Epi and trans-illumination

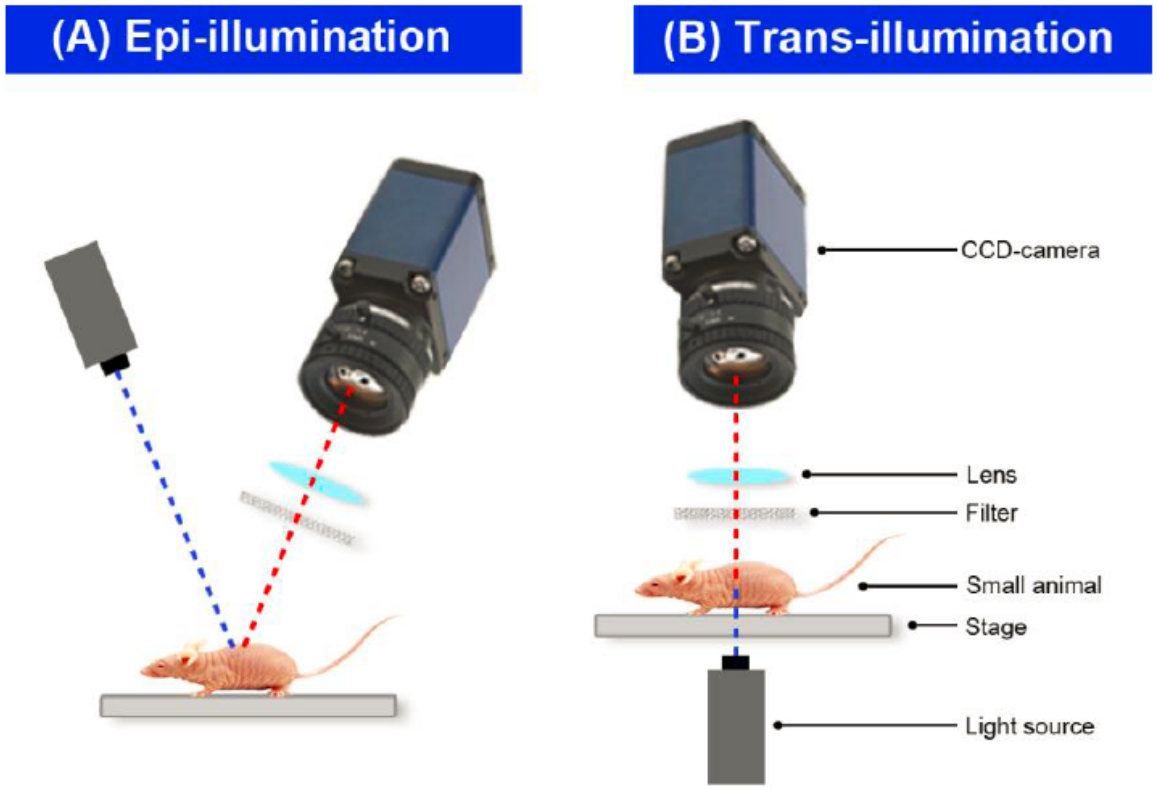
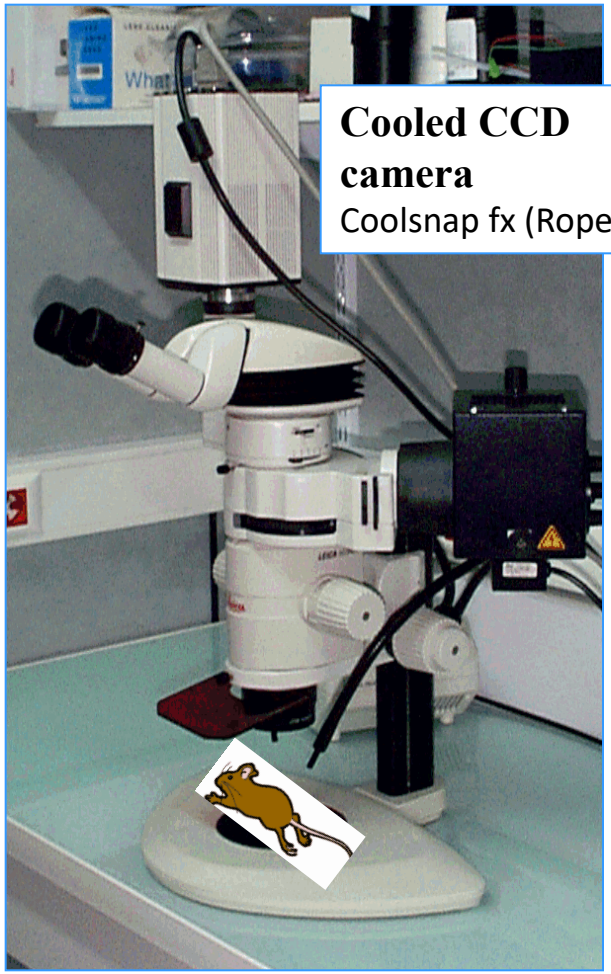
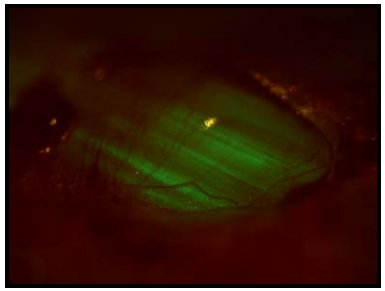


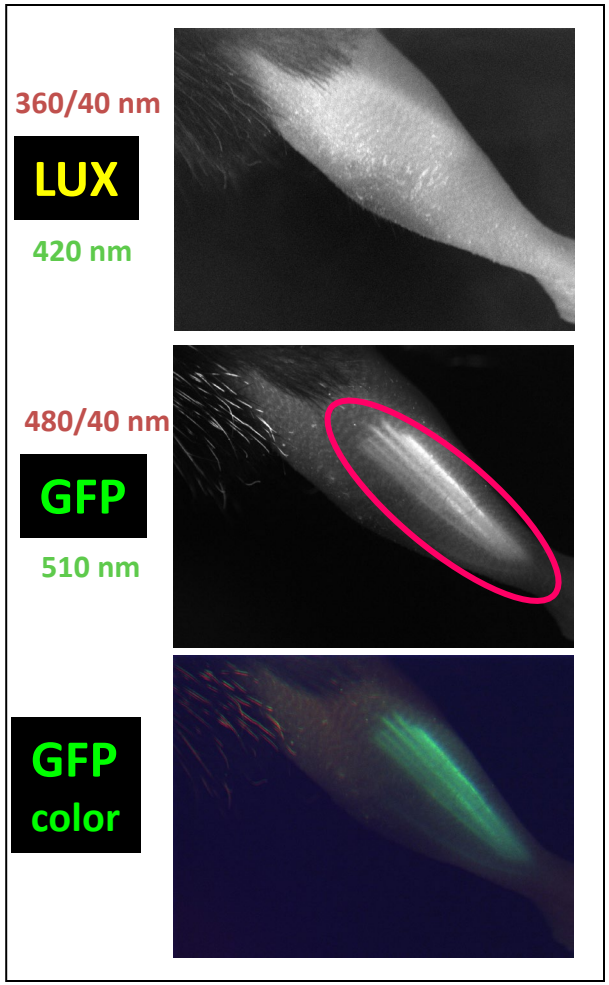
Figure 3: Basic principle of epi-illumination (A), in which the light source and detectors are positioned on the same side of the animal, and trans-illumination (B), in which the animal is illuminated from one side and the emitted photons that have propagated through the tissue are detected on the other side.

Fluorescence stereo microscope: noninvasive imaging of part or whole animal

Noninvasive fluorescence monitoring - expression of GFP in mouse muscle

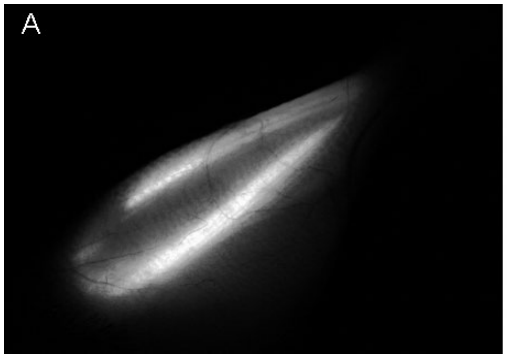


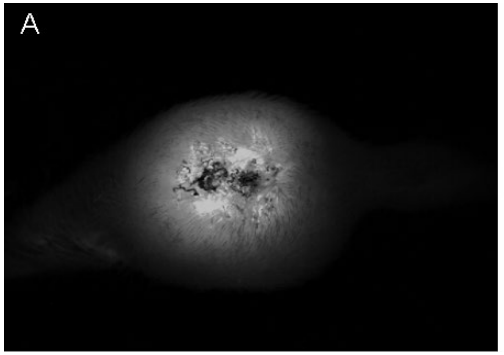




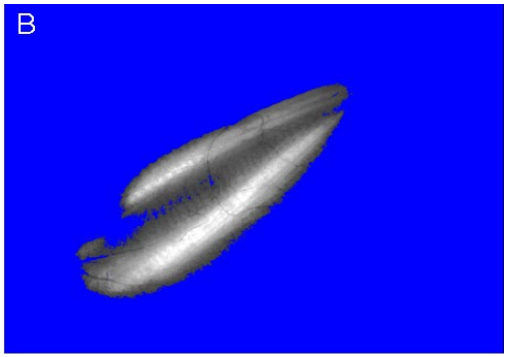
Fluorescence stereo microscop

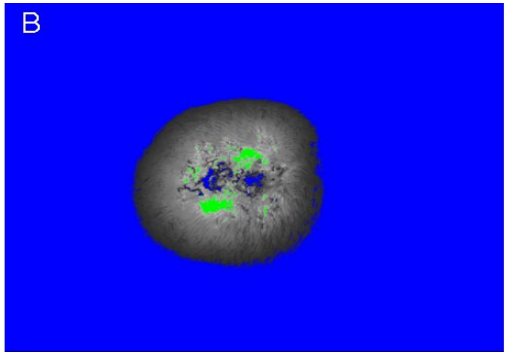
Fluorescence images of muscle and tumor



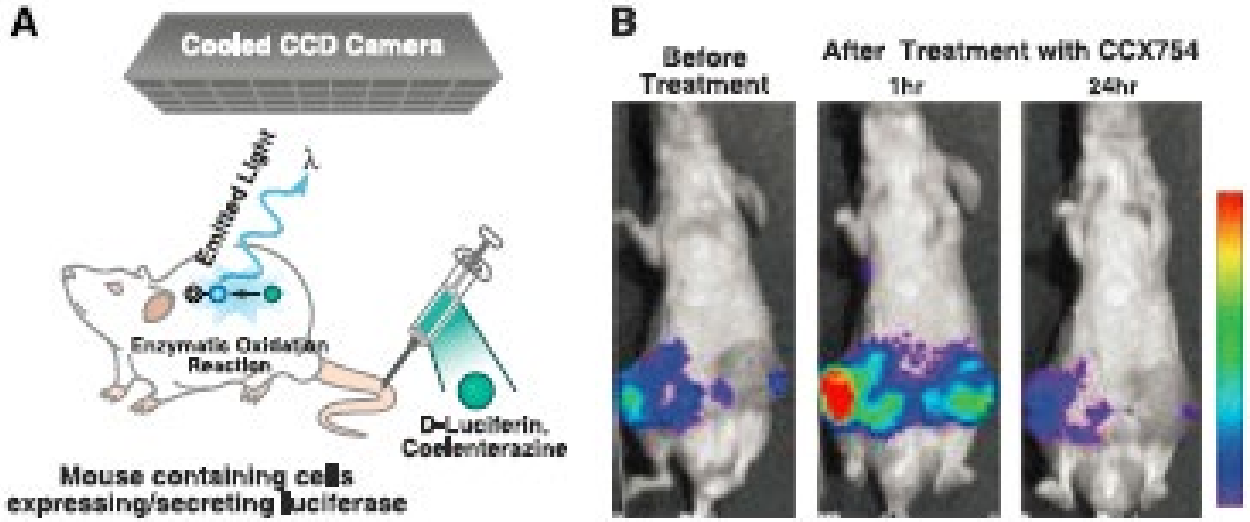


Determination of average intensity





Small animal optical bioluminescence imaging



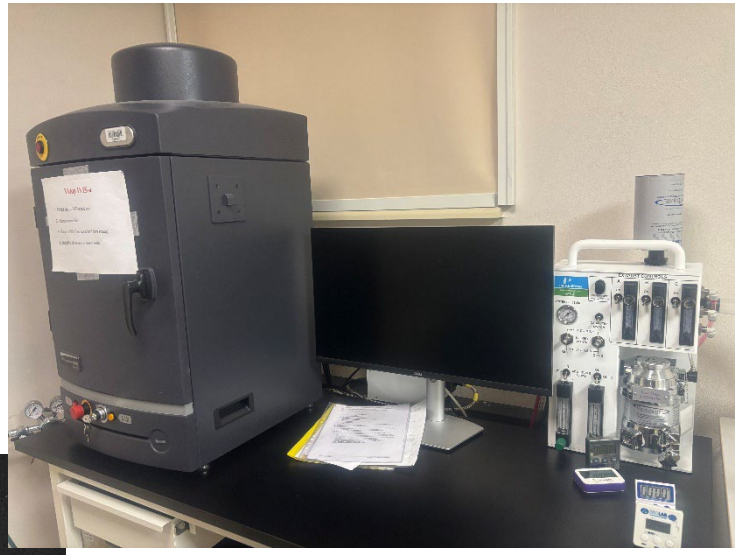
Advantages

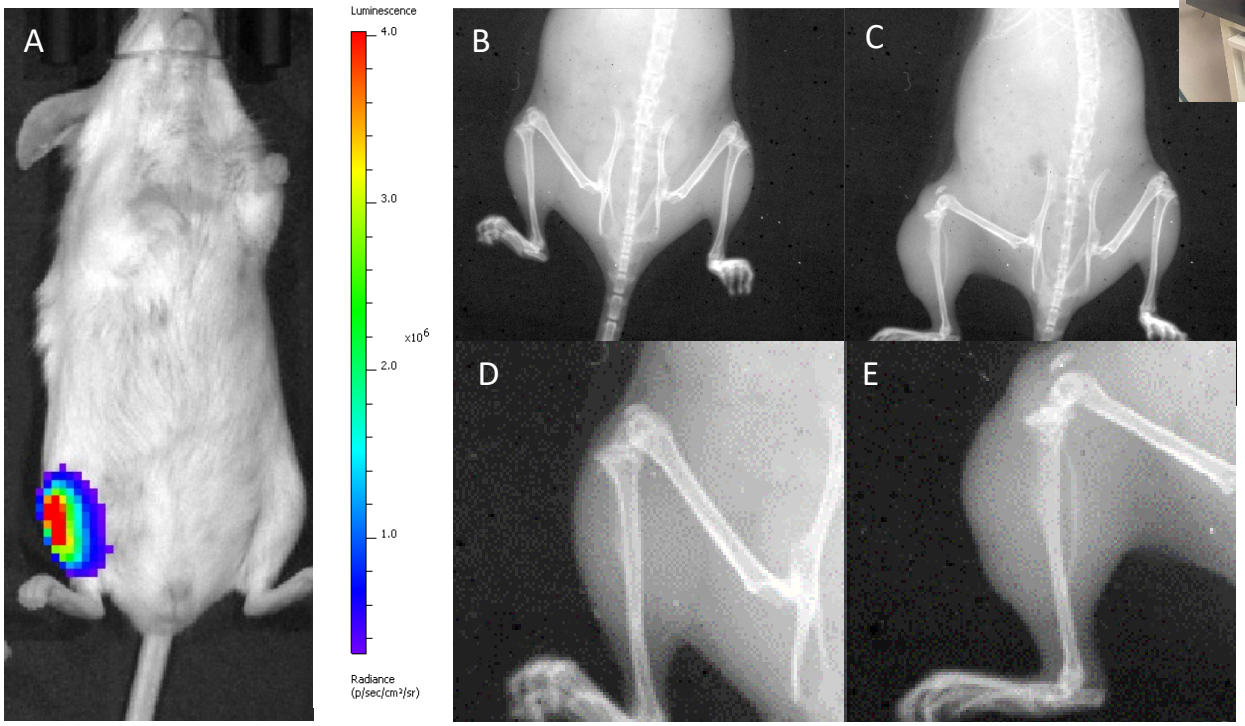
- Relatively inexpensive
- Excellent sensitivity
- Good temporal resolution
- User friendly
- Multiplexing capabilities
- No endogenous bioluminescence

Drawbacks

- Limited depth of penetration
- Poor spatial resolution at greater depths
- Images are relatively surface-weighted
- Substrates and enzymatic co-factors requir
- Tomography challenging
- Clinical translation very limited

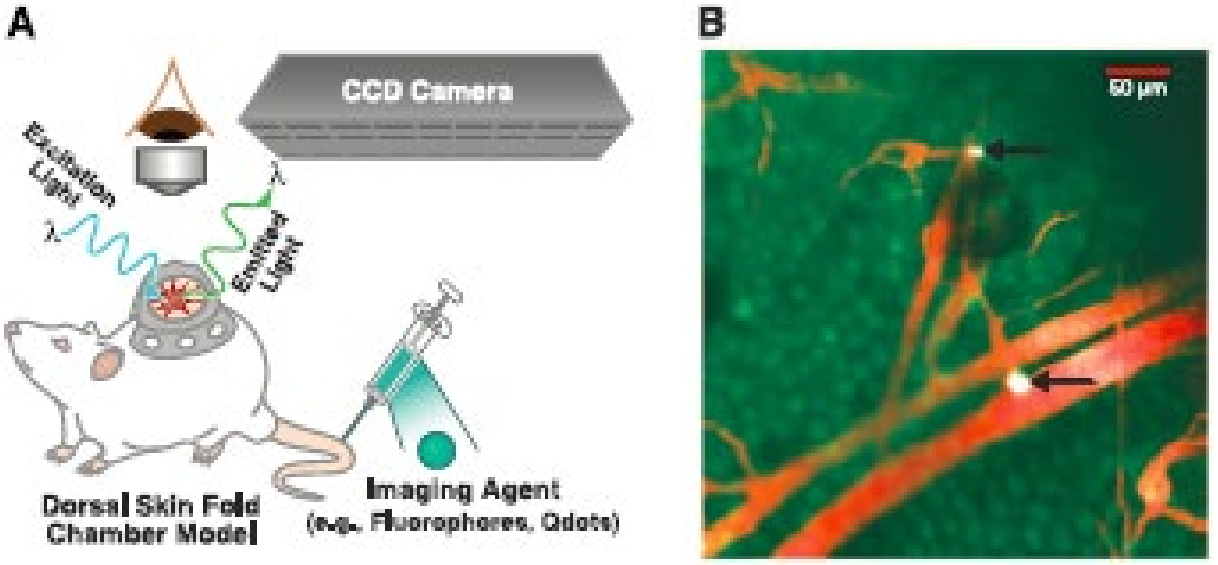
IVIS - in vivo imaging sistem: fluorescenca, luminiscenca, rentengensko slikanje





IVIS imaging of orthotopic osteosarcoma at day 6 after implantation (A) and x-ray images of the animals and knee close-up at day 6 after implantation (B,D) and at day 25 where bone lysis is evident (C, E).

Small animal intravital microscopy (IVM)



Advantages

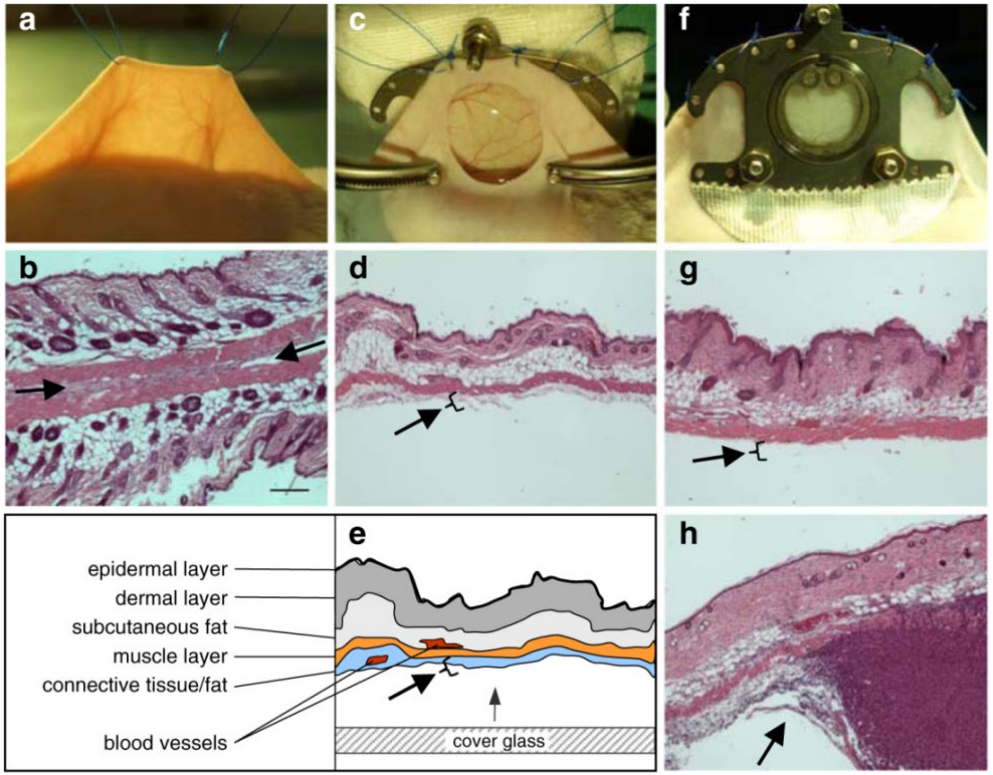
- Excellent spatial distribution
- Multiplexing capabilities
- Yields quantitative measures of cell size and motility
- Dynamic information about microscopic cellular events

Drawbacks

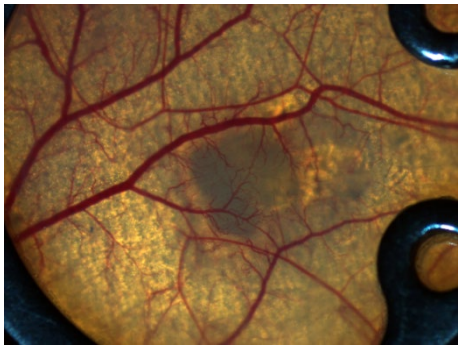
- Poor depth of penetraton
- Small field of view
- Can require multiple laser excitations
- Animal models are limited

Intravital microscopy

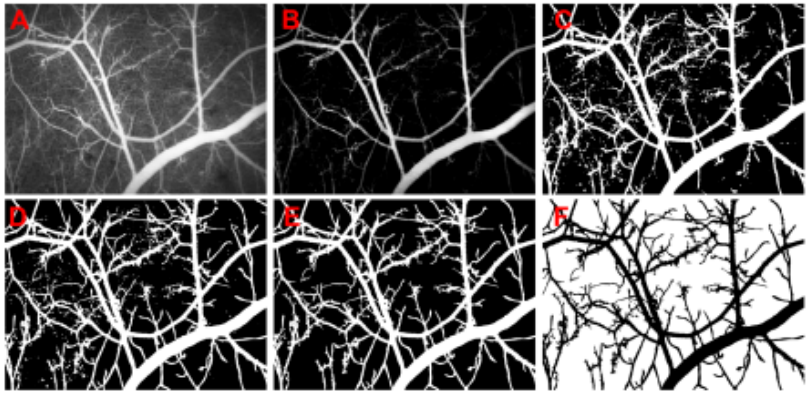
- Direct visual access to the blood vessels and surrounding tissue
- Repetitive observations of the same animal
- In combination with modern microscopy techniques it enables high spatial and time resolution of imaging



Koehl GE. et al, Clin Ex Metastasis, 2009

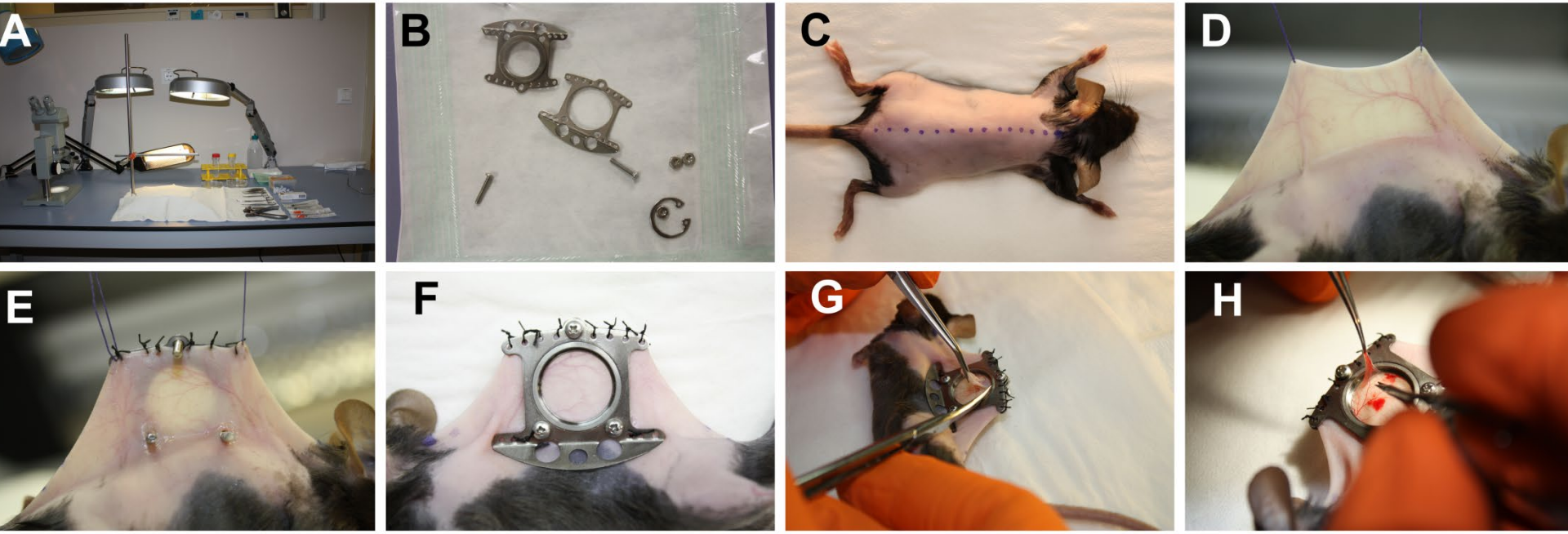


Tumor grown in dorsal window chamber



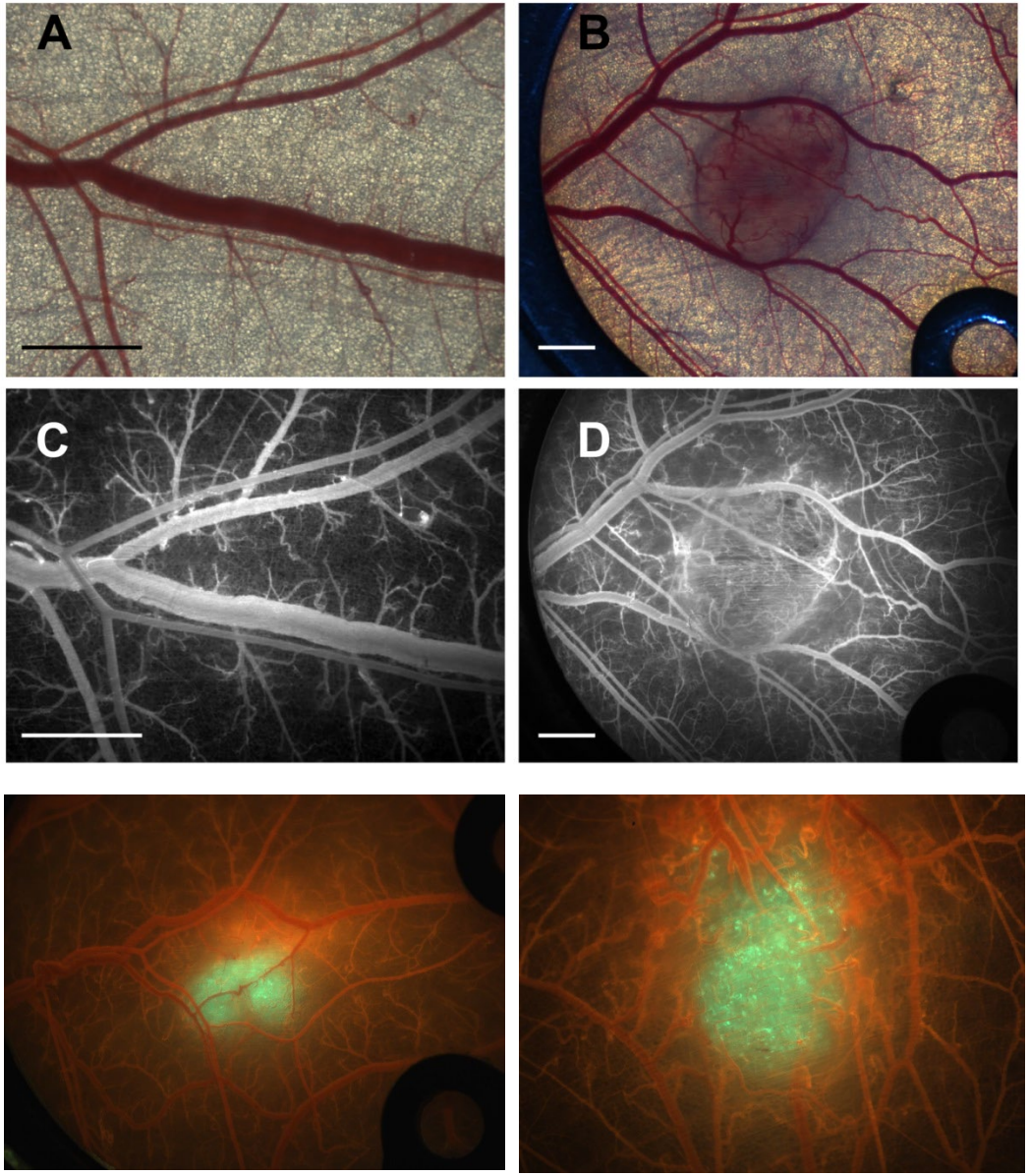
Masks of blod vessels: calculation of fluorescence intensity inside the vessles and in extravascular tissue

Surgical implantation of dorsal skinfold window chamber in mice

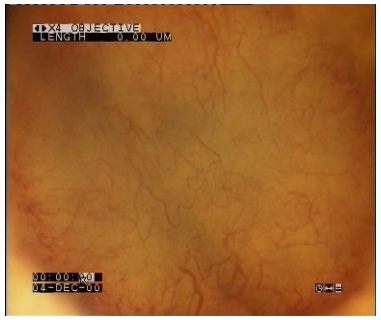


Dorsal skinfold window chamber

- Angiogenesis
- Blood flow modification
- Tumor formation
- Vascular therapies
- Treatment effectiveness
- Metastasis
- …

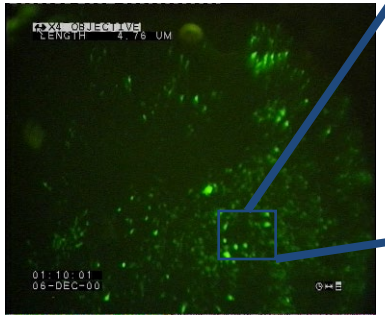


Detection of transfection effciency by determining the number of GFP expressing cells in tumors

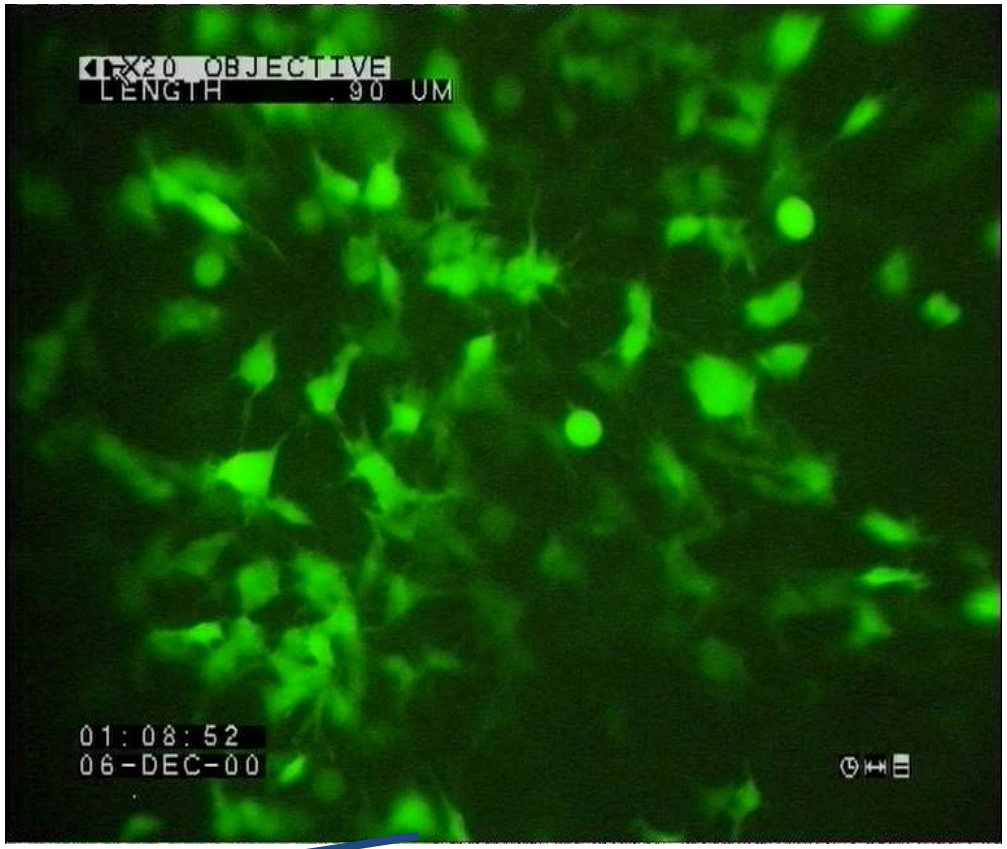


Pre x 4

Pre EP epi x 4



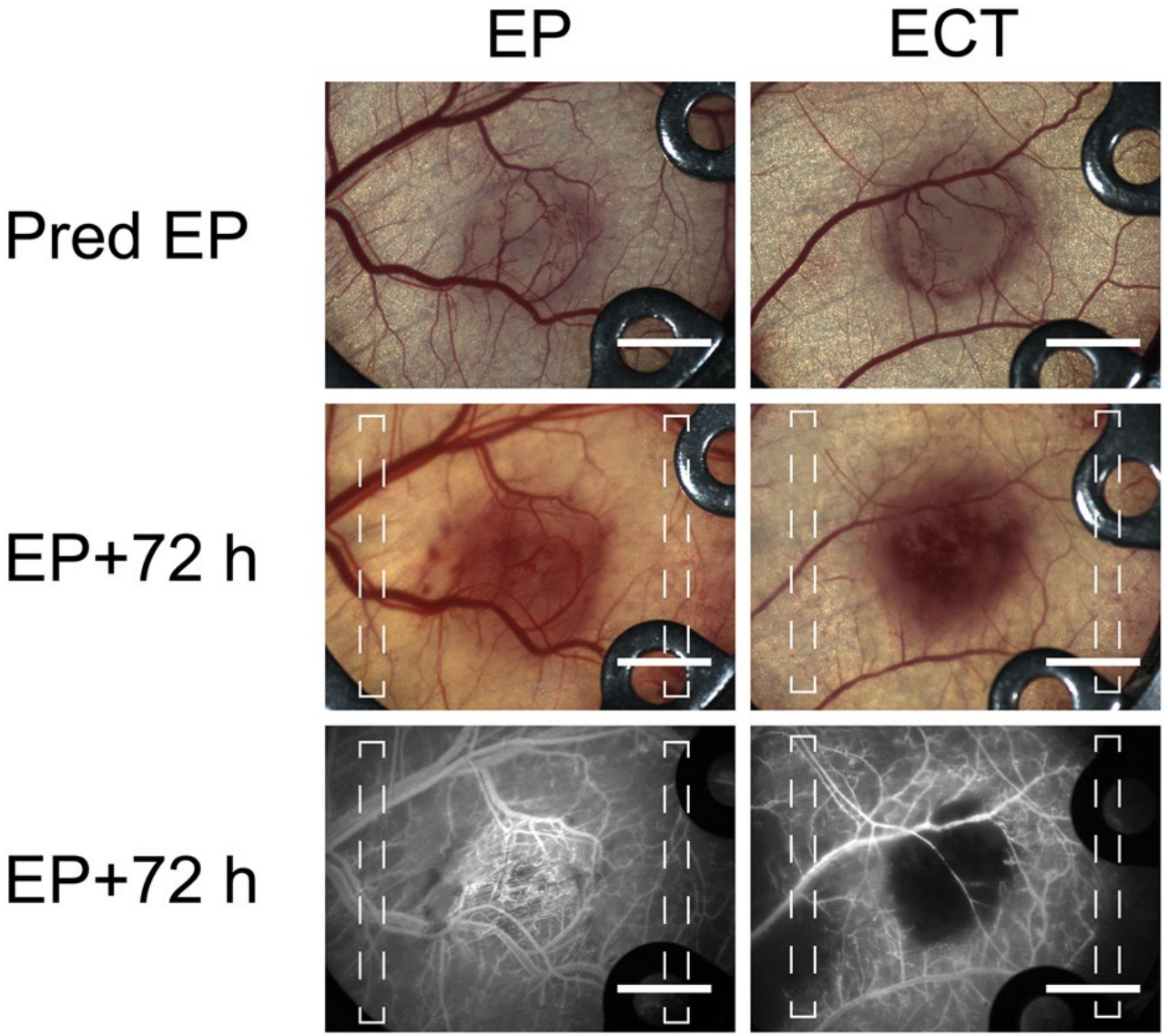
2 days epi x 4



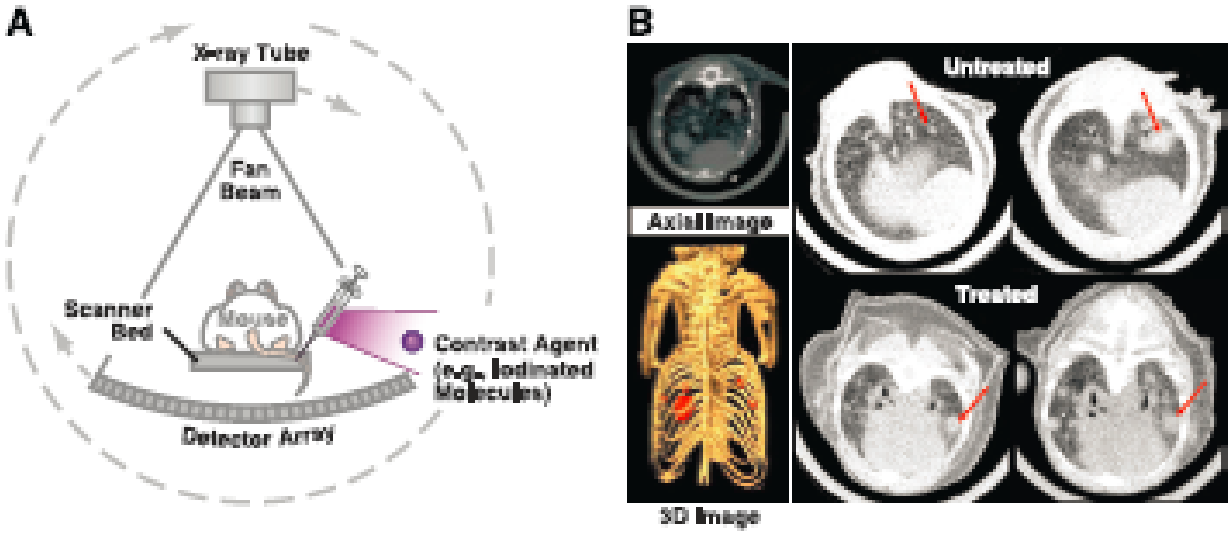
2 days epi x 20

Rat No. 7

Electrochemotherapy does not affect normal blood vessels surrounding tumor



Smal animal computed tomography - CT



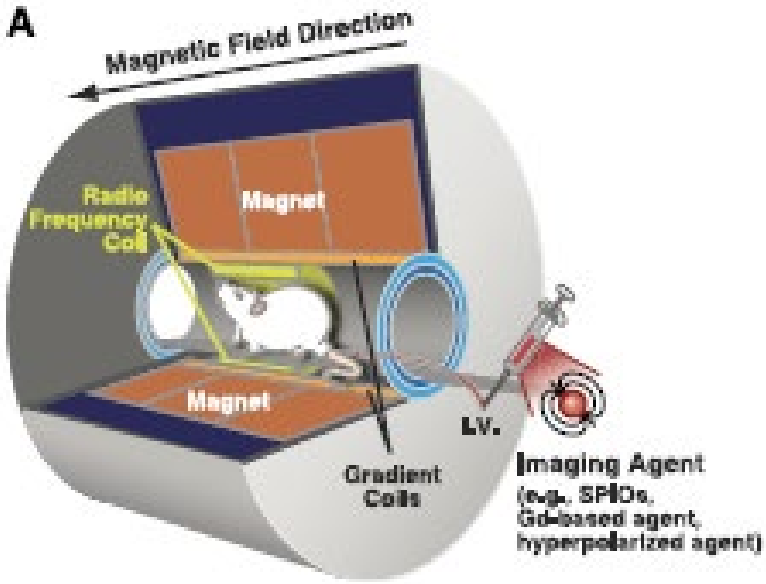
Advantages

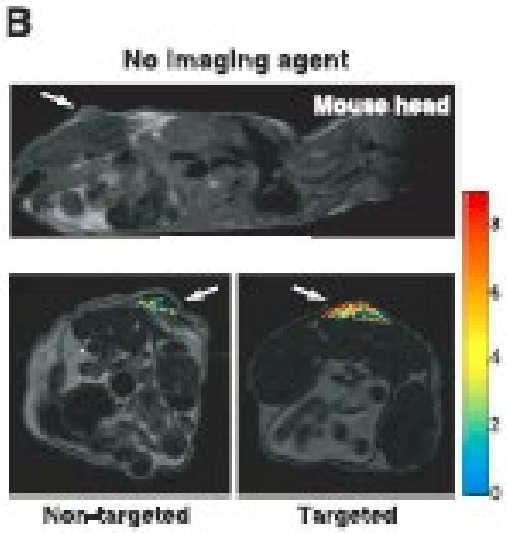
- Limitless depth of penetration
- High spatial resolution
- Good temporal resolution
- Clinical utility

Drawbacks

- Poor sensitivity (requires large mass of imaging agent)
- Primarly anatomic information
- Limited soft tissue resolution
- Limited molecular imaging applications
- Ionizing radiation

Small animal magnetic resonance imaging - MRI





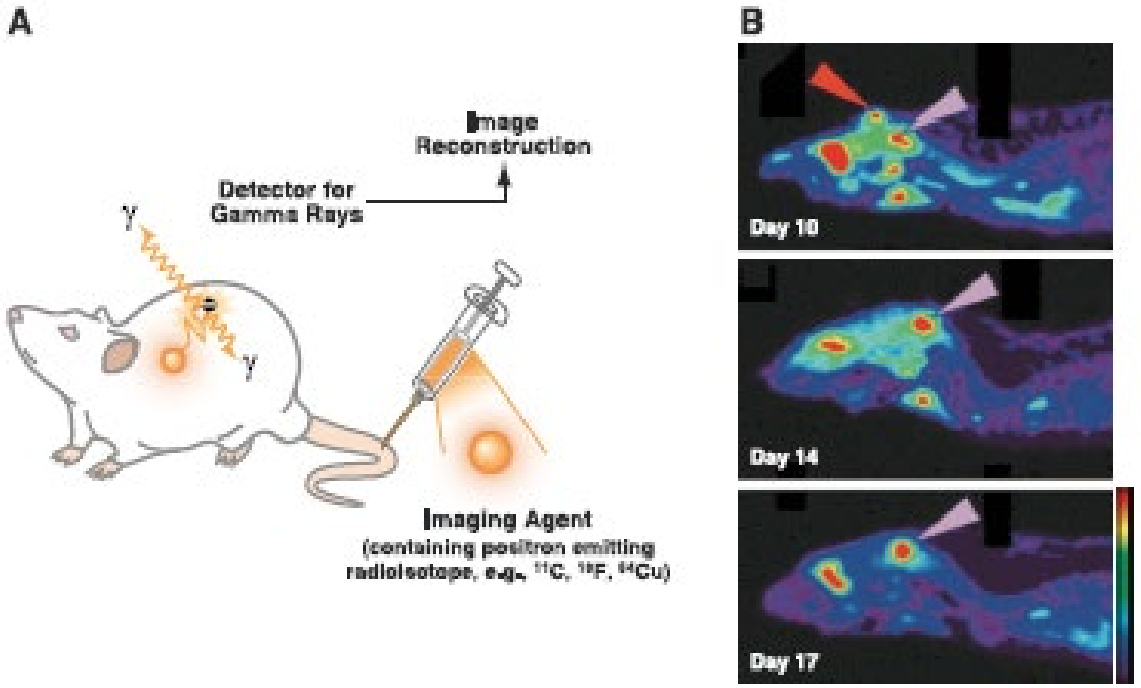
Advantages

- Limitless depth of penetration
- High spatial resolution
- Quantitative data no ionizing radiation
- Clinical utility

Drawbacks

- Poor sensitivity (requires large mass of imaging agent)
- Relatively expensive
- Relatively poor temporal resolution

Small animal positron emission tomography -PET



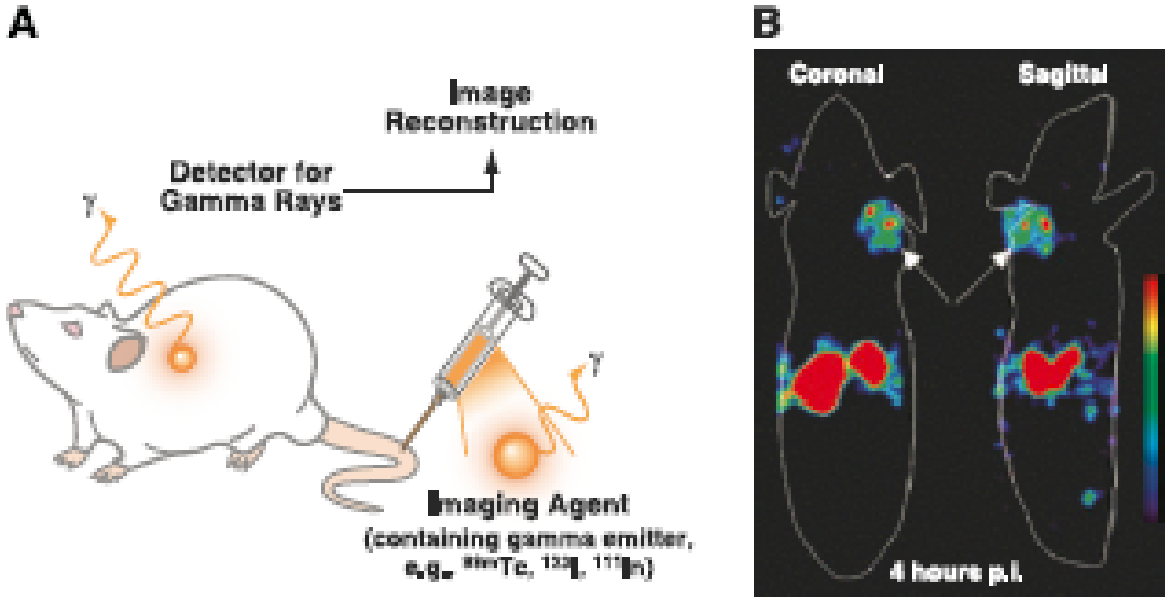
Advantages

- Limitless depth of penetration
- Excellent sensitivity
- Quantitative data
- Clinical utility

Drawbacks

- Relatively expensive
- Requires cyclotron/generator
- Limited spatial resolution
- Ionizing radiation

Small animal single photon emission computed tomography - SPECT



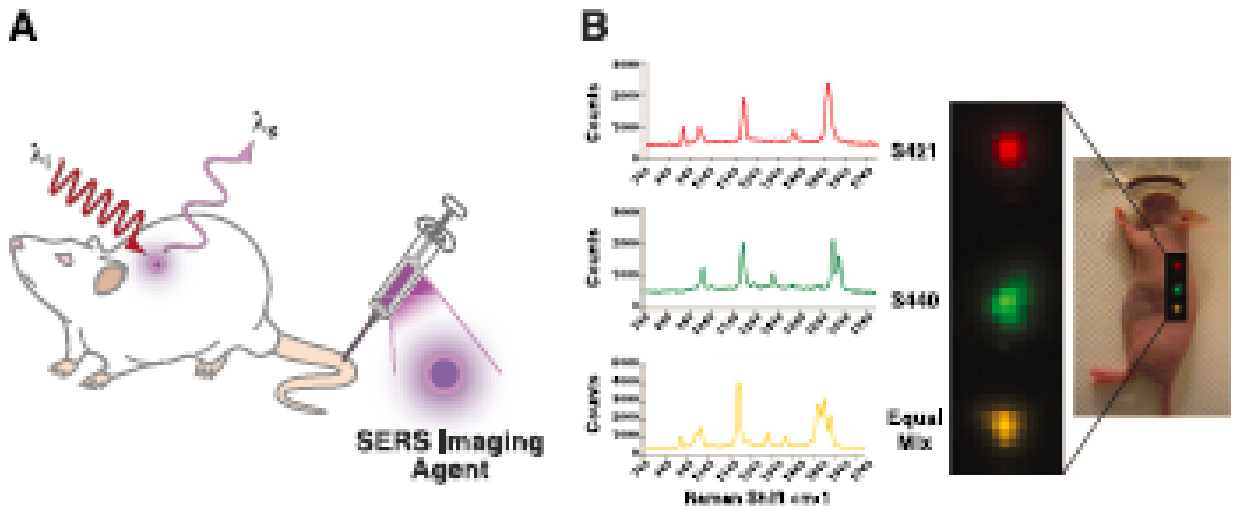
Advantages

- Limitless depth of penetration
- Excellent sensitivity
- Clinical utility

Drawbacks

- Relatively expensive
- Requires cyclotron/generator
- Limited spatial resolution
- Lack of attenuation corrections
- (therefore only semi-quantitative

Small animal surface enhanced RAMAN scattering (SERS) imaging



Advantages

- High sensitivity
- Significant multiplexing capabilities
- Sustainable signal over long periods
- Clinical utility in limited applications

Drawbacks

- Poor depth of penetration
- Tomography challenging
- Imaging large field of view challengi

PREPARE smernice

- Sistematično načrtovanje raziskav na živalih za zmanjšanje tveganj in izboljšanje kakovosti.
- Pomaga raziskovalcem načrtovati poskuse
- Zmanjša zaplete in trpljenje živali
- Poveča kakovost raziskav

1. Organizacija
2. Ljudje in kompetence
3. Postopki na živalih

ARRIVE smernice

- Poročanje o raziskavah na živalih za povečanje preglednosti in ponovljivosti.
- Izboljšanje kakovosti poročanja
- Podpora etični uporabi živali
- Lažje vrednotenje rezultatov
- Transparentnost znanstvenega dela

References and further reading

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Thank you for your attention!

Boy, I would love to be his pet cat!