CANCERBEAT

National Cancer Institute (LT)

PROGRESS REPORT MEETING

RIGA

2022-11-08

National Cancer Institute
Vilnius, Lithuania

The only specialised oncology institution in Lithuania.

The Institute acts as the clinical cancer centre with research departments.

NACIONALINIS VĖŽIO INSTITUTAS
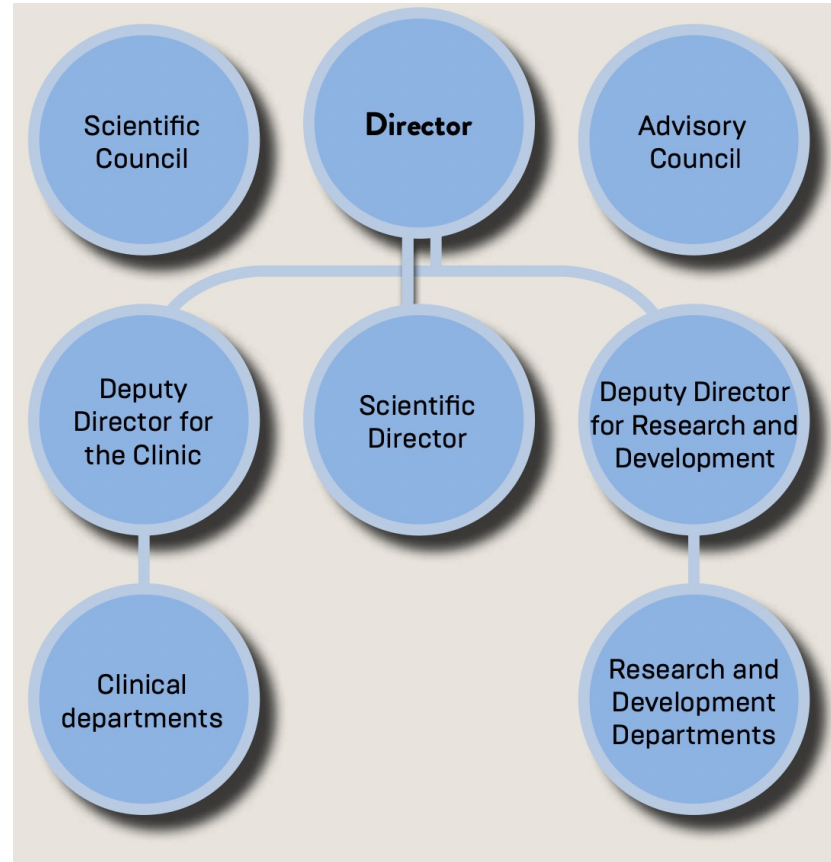NCI
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NCI Structure

Clinical departments
- Emergency Department
- Consultative Polyclinic Department
- Diagnostic Departments
- Surgical Departments
  - Anesthesiology & ICU
  - General and Abdominal Surgery
  - Thoracic Surgery
  - Head and Neck and Skin Cancer
  - Oncourology
  - Oncogynecology
  - Breast Surgery
- Therapeutic Departments
- Nursing Department
- Physical Medicine and Rehabilitation Department

2020:
131421 - consultations provided
13750 – patients treated

Scientific Council — Director — Advisory Council
Deputy Director for the Clinic — Scientific Director — Deputy Director for Research and Development
Clinical departments — Research and Development Departments

R&D Departments
- Biobank
- Clinical Trials Group
- Laboratory of Biomedical Physics
- Laboratory of Cancer Epidemiology
- Laboratory of Clinical Oncology
- Laboratory of Genetic Diagnostic
- **Laboratory of Immunology**
- Laboratory of Molecular Oncology
- Open Access Centre

2020:
- **3** doctoral theses
- **71** articles in WoS publications

2021:
- **5** doctoral theses
- **78** articles in WoS publications

Research & development programs @ NCI

SCIENTIFIC KNOWLEDGE AND INNOVATIVE TECHNOLOGIES FOR CANCER PREVENTION AND EARLY DETECTION

- Improvement of early prostate cancer detection
- Diagnostic and prognostic biomarkers in breast, renal, uterine cancer
- Nanomaterials in cancer theranostics
- Free circulating DNA in NSCLC
- Epigenetics and genome instability in colorectal cancer
- Isolation of tumor infiltrating lymphocytes
- Comorbidities in cancer patients
- New generation imaging technologies

INNOVATIVE MODELS AND SUSTAINABLE SOLUTIONS FOR CANCER THERANOSTICS, DIAGNOSIS, TREATMENT, AND IMPROVING THE QUALITY OF PATIENTS' LIFE

- Predictive biomarkers of chemotherapy response in ovarian and gastric cancer
- Mobile technologies with AI for cancer monitoring Immunotherapy in cancer patients
- Molecular tools for long-term monitoring and precision treatment in prostate cancer
- Circulating miRNAs for evaluation of treatment efficacy
- Fertility preservation program for cancer patients
- Cord blood NK cell research

Laboratory of Immunology

Prof Vita Pašukonienė

Neringa Dobrovolskienė, PhD

Karolina Žilionytė, PhD

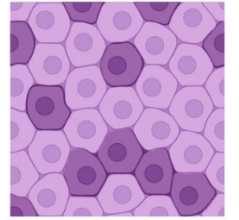**Agata Mlynska, PhD**
Nijolė Matusevičienė, MSc

Jan Aleksander Krasko, PhD
Olha Karaman, PhD
Emilija Paberalė, MSc
Eglė Žymantaitė, MSc

Comprehensive outlook on cancer immunology and immunotherapy

In vivo
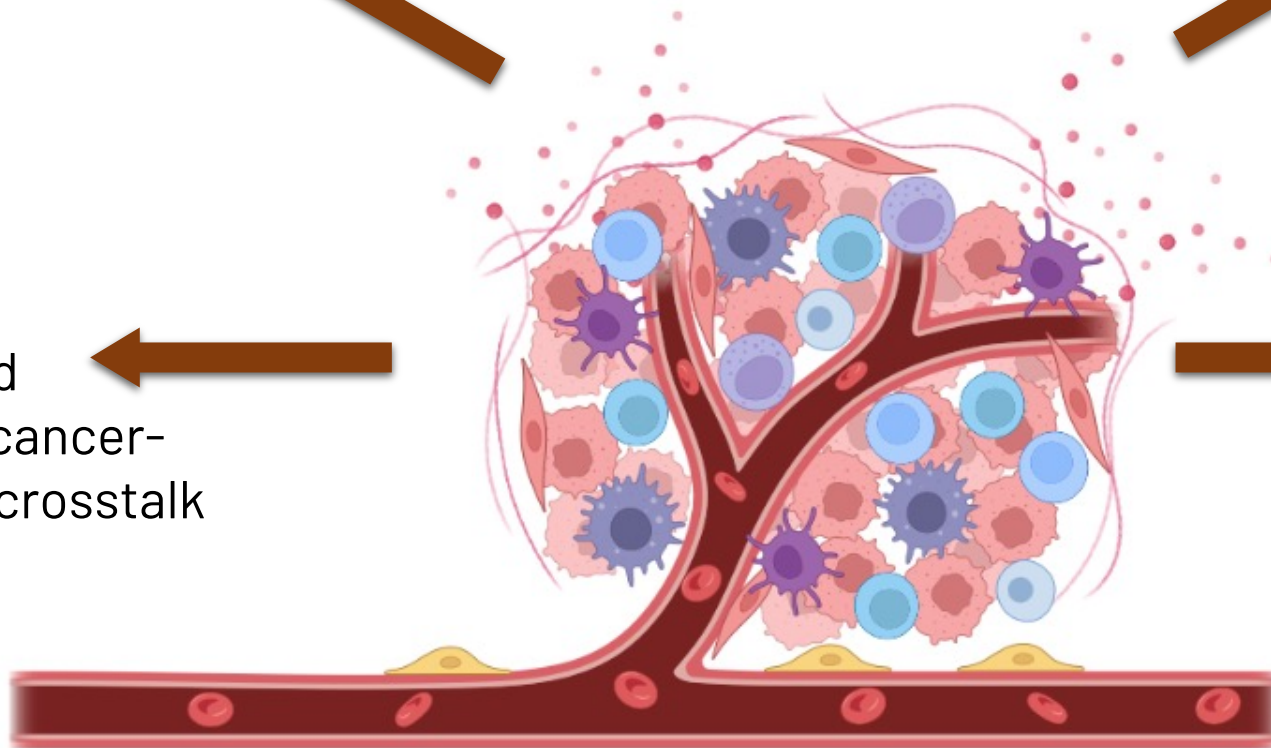Mechanistic and therapuetic preclinical trials in mouse models

Ex vivo
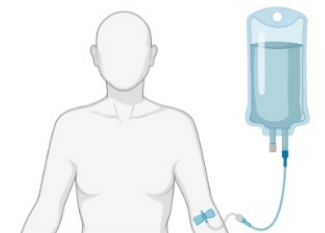Immune tumor subtyping and biomarker profiling

In vitro
Modelling and targeting of cancer-immune cell crosstalk

Therapy
Research, development, and clinical trials of advanced therapy medicinal products for cancer management

In vitro: Modelling and targeting of cancer-immune cell crosstalk

Area of interest and expertise

- Interplay between macrophages and ovarian/colorectal cancer cells

- Innovative delivery methods of theranostic nanomaterials into cancer and immune cells

- Adapting cancer-derived organoid technology for modelling tumor microenvironment

Ongoing projects

- Converting molecular profiles of myeloid cells into biomarkers for inflammation and cancer COST 2021-2025

- Modulating the microenvironment of immune-excluded tumors, Lithuanian Research Council 2022-2026

- Mesenchymal stem cells as vehicles for targeted delivery of theranostic nanoparticles into aggressive type of cancer cells, Lithuanian Research Council 2022-2025
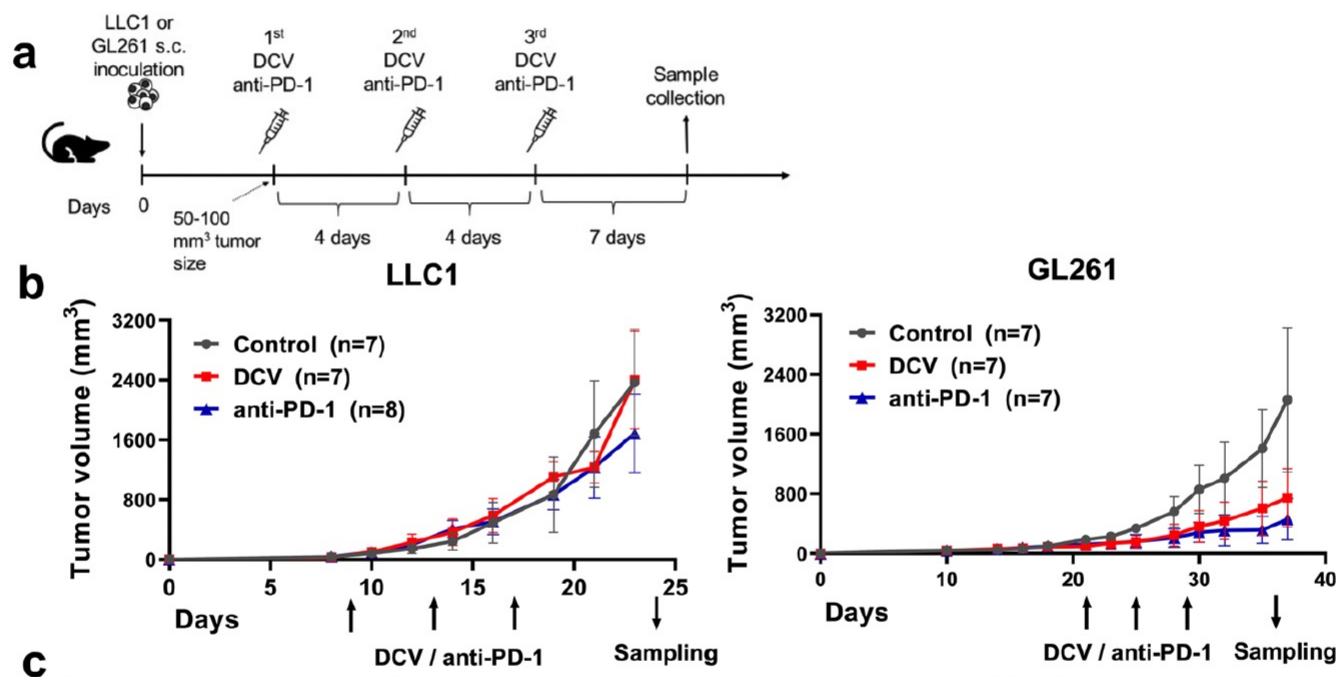
Publications

1 PMID33402483   2021

Metabolic-targeted Combination Therapy With Dichloroacetate and Metformin Suppresses Glioblastoma Cell Line Growth In Vitro and In Vivo.

Korsakova L, Krasko JA, Stankevicius E • In Vivo

2 PMID29904979   2018

Platinum sensitivity of ovarian cancer cells does not influence their ability to induce M2-type macrophage polarization.

Mlynska A, Povilaityte E ... Pasukoniene V • Am J Reprod Immunol. 2018 Sep

In vivo: Mechanistic and therapuetic preclinical trials in mouse models

Area of interest and expertise

- Investigating the role of antigen processing and presentation mechanism in tumor development and response to treatment

- Chemoimmunotherapy preclinical trials

Publications

1 PMID35478153   2022

Thymus Subset Alterations Accompanying Concomitant Tumor Immunity Mimics Phenotypic Patterns of Cytotoxic Drug Doxorubicin.

Zaleskis G, Characiejus D ... Pasukoniene V • In Vivo

2 PMID35467108   2022

Correction to: Functional antigen processing and presentation mechanism as a prerequisite factor of response to treatment with dendritic cell vaccines and anti-PD-1 in preclinical murine LLC1 and GL261 tumor models.

Žilionytė K, Bagdzevičiūtė U ... Pašukonienė V • Cancer Immunol Immunother

3 PMID33952461   2021

Comparative Evaluation of Cellular Uptake of Free and Liposomal Doxorubicin Following Short Term Exposure.

Zaleskis G, Garberyte S ... Pasukoniene V • Anticancer Res

In vivo: Mechanistic and therapuetic preclinical trials in mouse models

Area of interest and expertise

- Developing models of physical activity and breast cancer

- Dynamics and kinetics of doxorubicin uptake and accumulation in tissue

Ongoing projects

- Exploring the molecular mechanisms behind the effects of physical exercise on breast cancer prevention, EEA and Norway Grants 2021-2024

- Molecular markers for predicting effective combinations of chemoimmunotherapy in mice with tumors of different immunogenicity, Lithuanian Research Council 2018-2022

- Selective absorption and sequestration of soluble and liposomal doxorubicin in tumor cells, NCI Research Grant 2021-2022

Publications

4 PMID33046971   2020

Doxorubicin uptake in ascitic lymphoma model: resistance or curability is governed by tumor cell density and prolonged drug retention.

Zaleskis G, Garberytė S ... Pašukonienė V • J Cancer

5 PMID31955885   2020

Salinomycin and dichloroacetate synergistically inhibit Lewis lung carcinoma cell proliferation, tumor growth and metastasis.

Skeberdyte A, Sarapiniene I ... Jarmalaite S • Biochem Biophys Res Commun. 2020

6 PMID29552144   2018

Post-operative unadjuvanted therapeutic xenovaccination with chicken whole embryo vaccine suppresses distant micrometastases and prolongs survival in a murine Lewis lung carcinoma model.

Kraśko JA, Žilionytė K ... Pašukonienė V • Oncol Lett

7 PMID27878261   2017

Bacterial ghosts as adjuvants in syngeneic tumour cell lysate-based anticancer vaccination in a murine lung carcinoma model.

Kraśko JA, Žilionytė K ... Pašukonienė V • Oncol. Rep.

# *Ex vivo:* Immune tumor subtyping and biomarker profiling

Area of interest and expertise

- Combining transcriptome and histology data for immune subtyping of immune desert, excluded, and inflamed tumors

- Systemic immune profiling for detection, prognostic, and predictive biomarker discovery

Ongoing projects

- Development of a tool for immune tumor subtyping and immunotherapeutic strategy individualization, European Social Fund 2020-2022

- Predictive biomarkers for intraperitoneal chemotherapy in patients with advanced ovarian cancer, NCI Research Grant 2018-2022

- Immunological differences between open and laparoscopic prostate cancer surgery, NCI Research Grant 2017-2022

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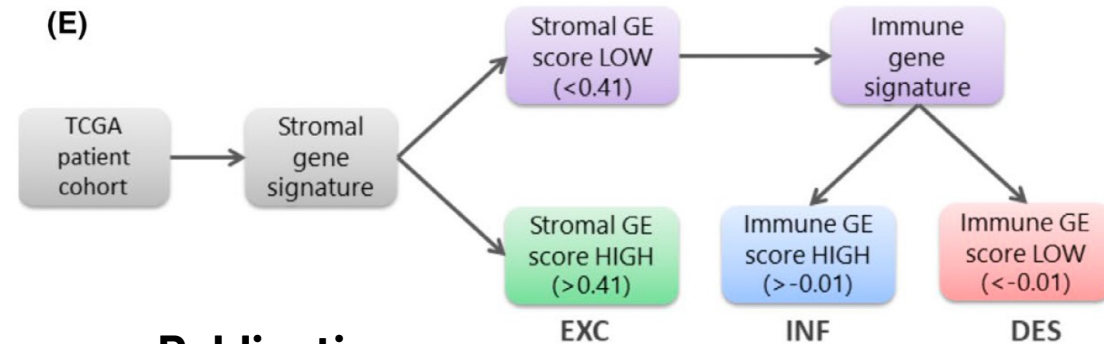
Publications

1 PMID34442004   2021

Immunophenotype Rearrangement in Response to Tumor Excision May Be Related to the Risk of Biochemical Recurrence in Prostate Cancer Patients.

Bosas P. Zaleskis G ... Jankevičius F • J Clin Med

2 PMID33744875   2021

A Refinement of Clinical Tumor Marker Monitoring: Why Not Use an Inverse Value of Doubling Time?

Zaleskis G, Bosas P ... Pašukoniene V • Med Princ Pract

3 PMID32294293   2020

A gene signature for immune subtyping of desert, excluded, and inflamed ovarian tumors.

Mlynska A, Vaišnorė R ... Pašukonienė V • Am J Reprod Immunol

4 PMID30483809   2019

Chemokine profiling in serum from patients with ovarian cancer reveals candidate biomarkers for recurrence and immune infiltration.

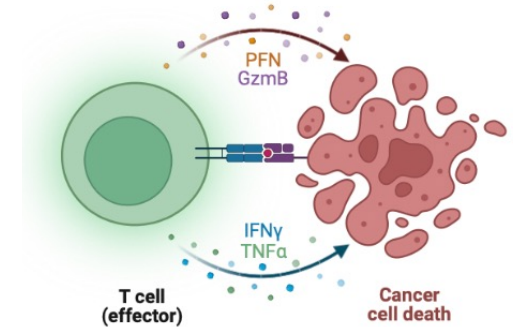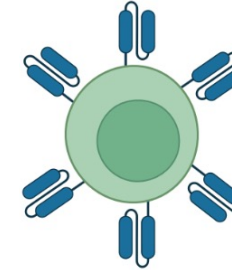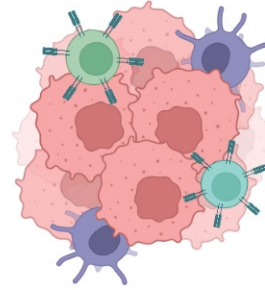Mlynska A, Salciuniene G ... Pasukoniene V • Oncol Rep. 2019 Feb
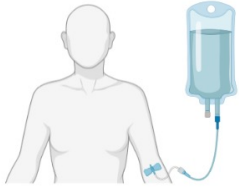
Therapy: Research, development, and clinical trials of advanced therapy medicinal products for cancer management

Dendritic cell vaccines
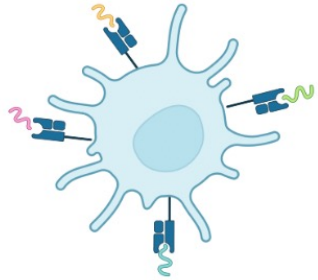
Developing T cell therapies:

TIL, CAR-T, CIK

Ongoing projects

- A study of the safety of chemoimmunotherapy with autologous Dendritic Cell Preparations in patients with stage III ovarian cancer EUDRACT 2020-003166-39, EU Funds, JSC Froceth 2018-2023

- Development of innovative high-throughput *in vivo* screening technology for validation of oncology drug candidates using a novel cellular double barcoding technology, EU Funds, JSC Froceth 2021-2023

- Combining cytokine-induced killer T cells and bacterial lectins for breast cancer treatment, Lithuanian Research Council 2022-2023

- The effect of immunotherapy on the survival of cancer patients at NCI, NCI Research Grant 2021-2024

Publications

1 PMID34413168 • PMC8378371   2021

Do the benefits of being a smoker hint at the existence of PD-1/PD-L1 sensitizers for patients on single-agent immunotherapy?

Zaleskis G, Pasukoniene V ... Urbonas V • J Immunother Cancer

2 PMID29895501   2018

Tumor lysate-loaded Bacterial Ghosts as a tool for optimized production of therapeutic dendritic cell-based cancer vaccines.

Dobrovolskiene N, Pasukoniene V ... Strioga M • Vaccine. 2018 Jul 5

3 PMID27864613   2017

Oncolysate-loaded Escherichia coli bacterial ghosts enhance the stimulatory capacity of human dendritic cells.

Michalek J, Hezova R ... Kudela P • Cancer Immunol. Immunother.

CANCERBEAT:
updates from National Cancer Institute (LT)

2022-11-08

Our team

Agata Mlynska, PhD

Prof. Vita Pašukonienė, PhD

Neringa Dobrovolskienė, PhD

Karolina Žilionytė, PhD student

Nijole Matusevičienė, animal tech

In collaboration with:

Vilnius University (animal facility)

Lithuanian Sports University (consultations on animal training plan)

Our role

- WP4. Effects of exercise-induced exosomes on breast cancer *in vivo*
- WP1. Analysis of the immune tumor microenvironment in patients' breast tumors.
- Help in other WPs where necessary

WP4: Effects of exercise-induced EVs on BC *in vivo*

Objective: To elucidate the effects of exercise-induced EVs on the immune tumour microenvironment and progression of metastatic cancer using a mouse model of BC.

Tasks:

4.1. Design and establish the periodic voluntary wheel running training plan from healthy BALB/c and C56BL/6 mice for subsequent exercise-induced EVs collection (NCI, OUH).

4.2. Exercise-induced EVs isolation and characterization (quantity, size, surface markers) (NCI, OUH).

4.3. Establishing two transplantable metastatic murine breast cancer models: 4T1 in BALB/c and E0771 in C57BL/6 mice and subjecting them to prophylactic and therapeutic administration of EVs. Monitoring of tumor growth, periodic collection of tumor and blood samples (NCI).

4.4. Immune TME profiling will be done by flow cytometry, qPCR and multiplex immunofluorescence. Profiling of peripheral blood and serum samples will be done by flow cytometry and cytokine assays (NCI, BMC).

Outputs/deliverables: Evaluation of exercise-induced EVs influence on BC growth, metastatic potential and functional phenotype of tumour-infiltrating immune cells (1 publication; 2 conference theses)

WP4: Effects of exercise-induced EVs on BC *in vivo*

Objective: To elucidate the effects of exercise-induced EVs on the immune tumour microenvironment and progression of metastatic cancer using a mouse model of BC.

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4.1. Design and establish the periodic voluntary wheel running training plan from healthy BALB/c and C57BL/6 mice for subsequent exercise-induced EVs collection (NCI, OUH). – **DONE**

4.2. Exercise-induced EVs isolation and characterization (quantity, size, surface markers)(NCI, OUH). – ongoing

4.3. Establishing two transplantable metastatic murine breast cancer models: 4T1 in BALB/c and E0771 in C57BL/6 mice and subjecting them to prophylactic and therapeutic administration of EVs. Monitoring of tumor growth, periodic collection of tumor and blood samples (NCI). – ongoing

4.4. Immune TME profiling will be done by flow cytometry, qPCR and multiplex immunofluorescence. Profiling of peripheral blood and serum samples will be done by flow cytometry and cytokine assays (NCI, BMC). – ongoing

Outputs/deliverables: Evaluation of exercise-induced EVs influence on BC growth, metastatic potential and functional phenotype of tumour-infiltrating immune cells (1 publication; 2 conference theses)

4.1. Design and establish the periodic voluntary wheel running training plan from healthy BALB/c and C57BL/6 mice for subsequent exercise-induced EVs collection – **DONE**

Animal experiment approval granted in May 2021.

Training plan: 10 treadmill running sessions (30 min), with incremental increase of speed (average 12-14 m/min), classified as moderate cardio activity (60% of mouse capacity).
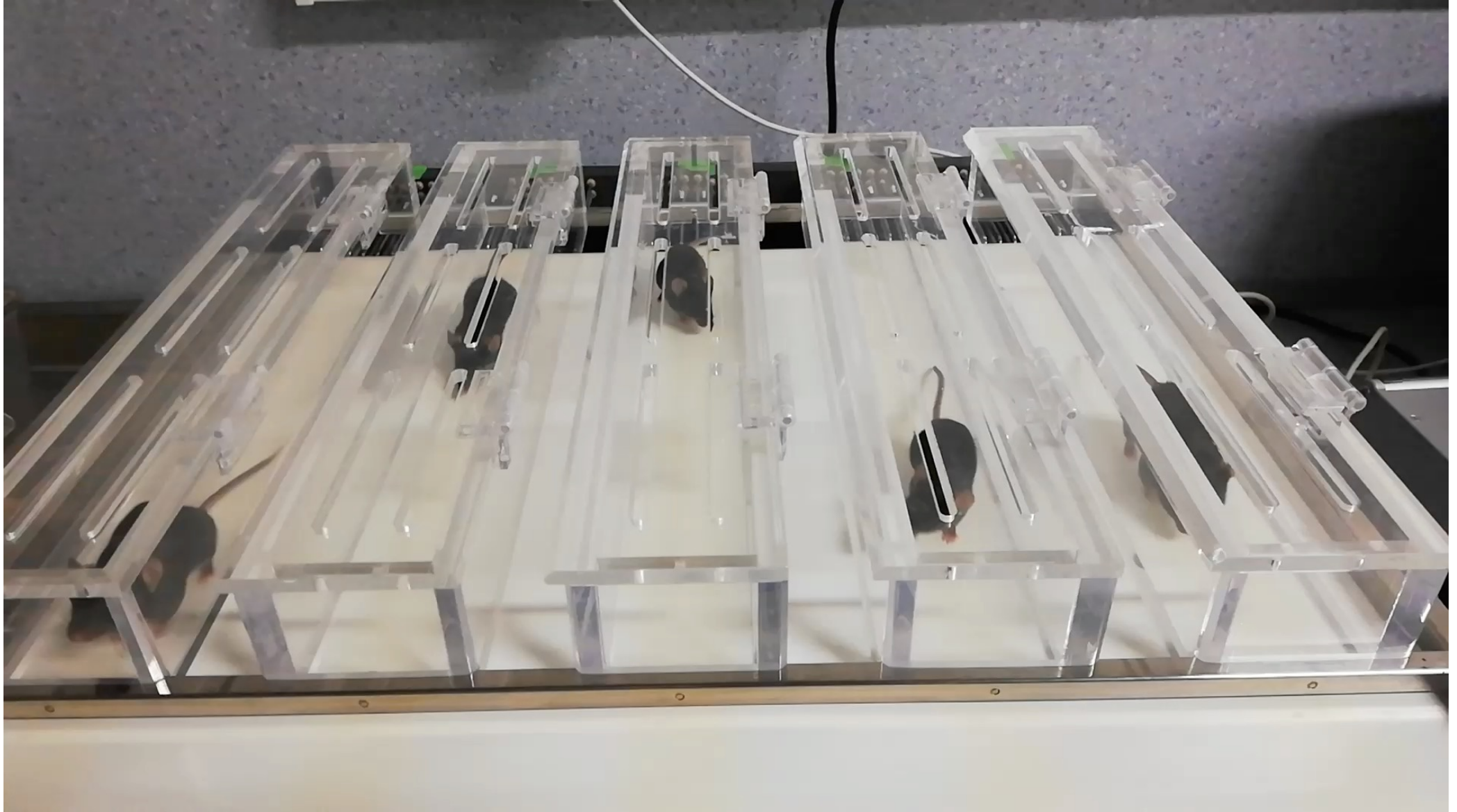
Designed based on LSU experience and published state-of-the-art (Adamovich et al, Cell STAR Protocols, 2021 and others)
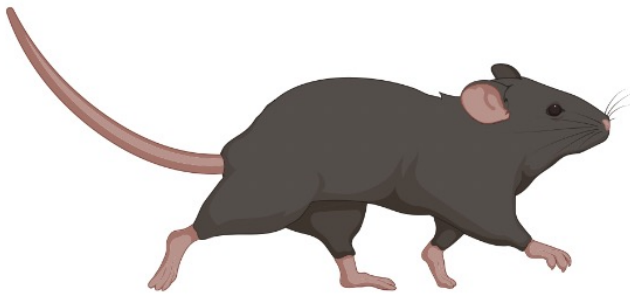
C57BL/6
N=30
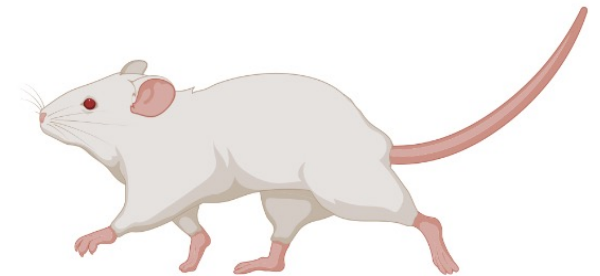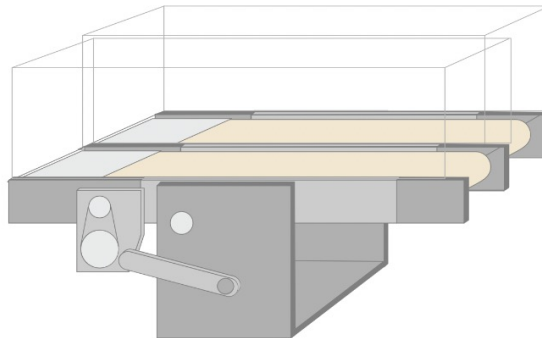
BALB/c
N=30

4.1. Design and establish the periodic voluntary wheel running training plan from healthy BALB/c and C57BL/6 mice for subsequent exercise-induced EVs collection – DONE

Training plan: 10 treadmill running sessions (30 min), with incremental increase of speed (average 12-14 m/min), classified as moderate cardio activity (60% of mouse capacity).

C57BL/6
N=30

BALB/c
N=30

3 * 30 min standard running sessions
    Blood drawing (up to 0,2 ml/mouse), 2 days recovery
5 * 30 min standard running sessions
    Blood drawing (up to 0,2 ml/mouse), 2 days recovery
2 * 30 min standard running sessions
    Terminal blood collection (as much as possible)
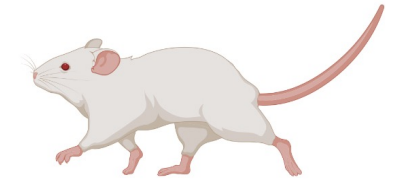
7 * 30 min standard running sessions
    Blood drawing (up to 0,2 ml/mouse), 2 days recovery
3 * 30 min standard running sessions
    Terminal blood collection (as much as possible)

Routine WBC testing to control overall health and allow plasma pooling.
Separation and -80 freezing of plasma samples for further EVs isolation and characterization

4.2. Exercise-induced EVs isolation and characterization
ongoing

After consulting with Aija and Alicia, we continued with IZON for EVs isolation

Rough estimation of recovered exosome fraction protein content in pilot experiment

Pitfalls:

- Non-optimal blood collection - SOLVED

- Low yield of EVs – hopefully SOLVED

- Lack of tools for thorough EVs characterization – team up with OUH and BMC
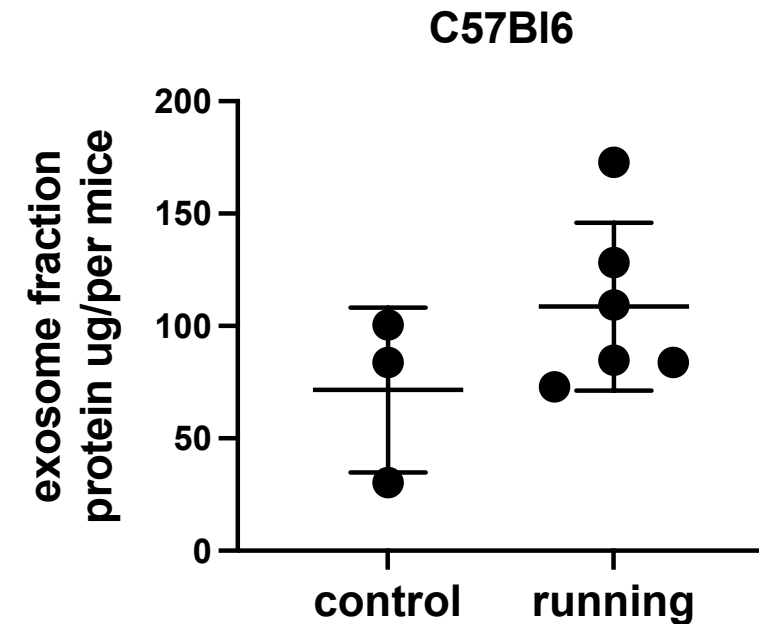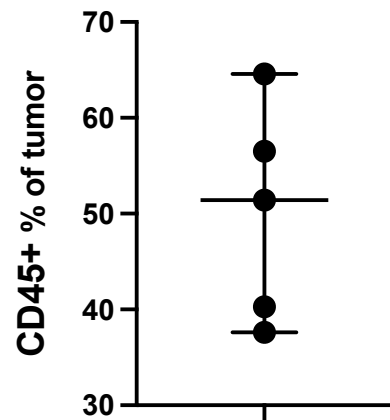

C57Bl6

4.3. Establishing two transplantable metastatic murine breast cancer models: E0771 in C57BL/6 mice (**DONE**) and 4T1 in BALB/c mice – ongoing

E0771 in C57BL/6 mice
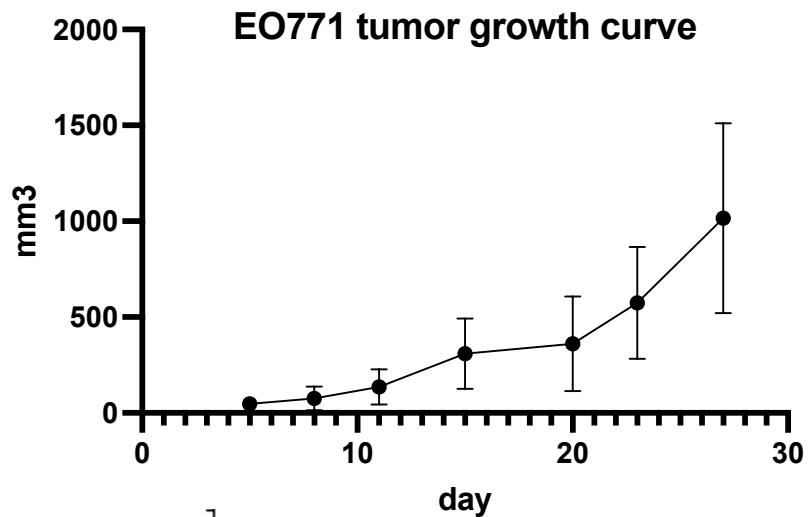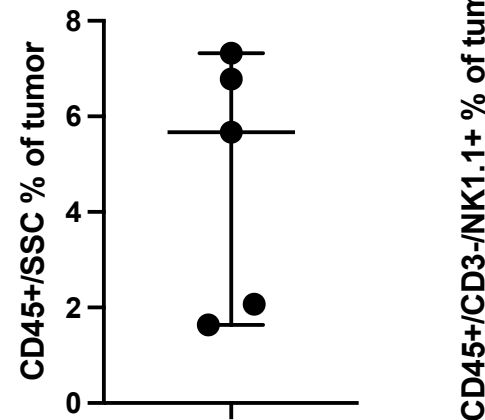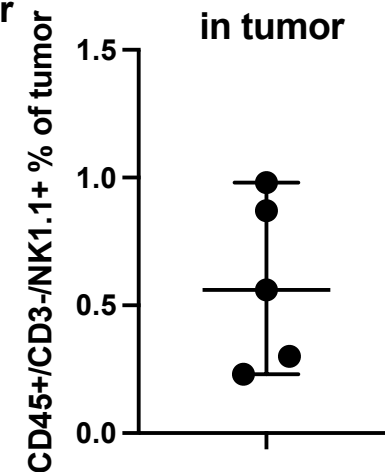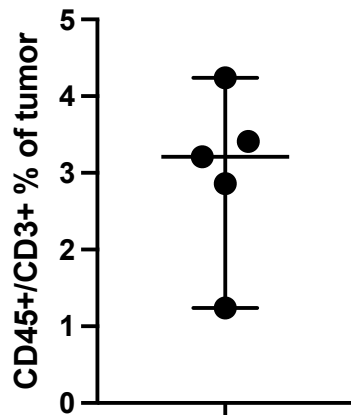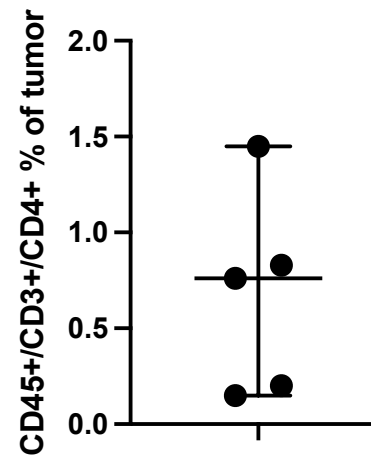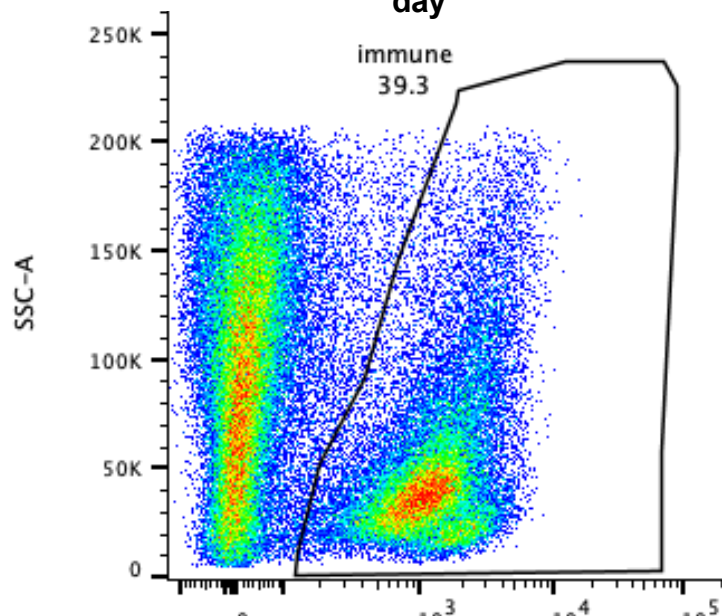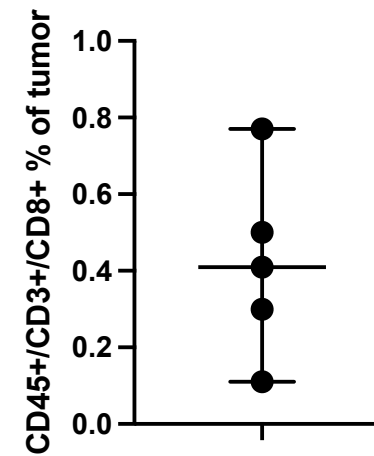
Further work

- All plasma samples foreseen for EV isolation are collected (based to quantitites used in other studies).
  - Isolation
  - Characterization
  - quantification
- One BrCa murine model set up successfully, another one should be characterized and ready by Jan 2023.
- Treatment group design.