Importance of Tumor Microenvironment in the Preclinical Estrogen Receptor Positive Breast Cancer – Primary Tumor and Bone Metastasis Models

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I have the following financial relationship to disclose:

Employee of Pharmatest Services Ltd.
Breast cancer, Hormone-dependency and Bone metastases

- High risk of bone metastases in postmenopausal women with hormone receptor-positive breast cancer
- Hormonal therapy e.g. tamoxifen and toremifene are used to lower amount of estrogen in the body in hormone-receptor positive breast cancer patients
Discordance rates for ER and PR were highest for bone metastases.
Disease itself and/or therapeutic treatments can impact bone.
Valid preclinical models needed for efficacy testing of therapeutic agents.
Aim of the present study

• To establish breast cancer primary and bone tumor models with focus on tumor microenvironment

• To compare hormone-dependency between primary and bone tumor

• To compare ER, PR and AR profiles between primary and bone tumor
Materials and Methods

- Intact athymic female nude mice, age 5-6 weeks (Envigo)
- Soy-free diet (Teklad, 2916, Envigo)
- Estradiol (E2) supplementation with slow-release implants 5 µg/day or placebo implants (PreclinApps Ltd.)
- MCF-7 cells (human breast cancer derived from metastatic site, ATCC) inoculated orthotopically into mammary fat pad (5 x 10^6) or intratibially (1 x 10^6)
- Study length 5-9 weeks
- Study readouts:
  - Orthotopic tumor: tumor size *in vivo* and histology
  - Tumor in bone: lesion type by radiography and histology
  - Serum E2 measurements by LC/MS-MS
  - Immunohistochemical receptor stainings for primary and bone tumors (BioSiteHisto Ltd):
    - Estrogen receptor alpha (ER, SP1, Spring Bioscience), progesterone receptor (PR, SP2) and androgen receptor (AR, SP107). FFPE samples, bone samples decalcified
Orthotopic MCF-7 tumor growth is dependent of E2 supplement

- Study terminated due to E2 related adverse effects in the urinary tract
  
  Collins et al. 2017; Dall et al., 2015; Kang et al. 2009; Pearse et al. 2009
MCF-7 tumor in bone grows also without E2 supplement

* Study group terminated due to E2 related adverse effects in the urinary tract
Tumor in bone induces osteoblastic and/or osteolytic lesions

- **Intact tibia**
- **Tumor-bearing tibia**

**No E2 supplement**

- **Intact (healthy) tibia**
- **Tumor-bearing tibia**

**With E2 supplement**

- **Intact* tibia**
- **Tumor-bearing tibia**

*Note direct effects of E2 in bone*
Receptor profile in orthotopic MCF-7 tumor: ER, PR and AR

- ER+
- PR+
- AR+

Tumor with E2 (HE)
Receptor profile in intratibial MCF-7 tumor with E2 supplement
Receptor profile in intratibial MCF-7 tumor *without* E2 supplement

- Intratibial tumor *without* E2 (HE)
- ER+
- PR-
- AR-/+
Summary

• Orthotopic MCF-7 tumor needs E2 supplement to support tumor growth

• In the bone, MCF-7 tumor growth does not need E2 supplement

• ER and AR expression are not dependent on the E2, however without E2 supplement loss of PR expression

• Focus in preclinical studies should be addressed also on metastatic site where cancer cells are e.g. under influence of bone microenvironment and receptor expression discordance may occur
Future directions
A new preclinical model for immuno-oncology: Combination of tumor, Bone microenvironment and Immune system

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Tumor-bearing bone in humanized CIEA NOG mouse (Taconic Biosciences)
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