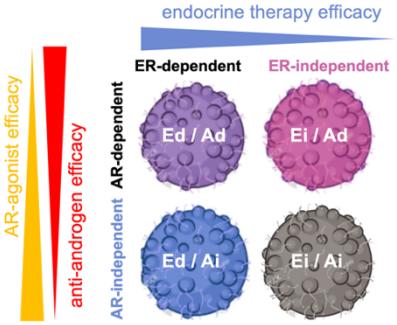
AR-agonist pre-treatment enables evolutionary steering in metastatic ER+ breast cancer

ER+ breast cancer is the most common subtype and accounts for most of breast cancer related deaths. Standard of care includes endocrine therapy (e.g. aromatase inhibitors, fulvestrant) in combination with CDK 4/6 inhibitors (e.g. ribociclib). However, resistance is a formidable challenge in metastatic breast cancer (mBC), as a majority of patients ultimately progress with refractory disease. Here, we investigate a novel treatment approach that takes into consideration evolutionary principles to pre-treat patients with safe, clinically-available drugs to enhance sensitivity to subsequent therapy. Designing treatment protocols that exploit tumor evolution and target acquired resistant traits is a novel approach to addressing resistance. We propose an **evolutionary double bind**: where an initial therapy elicits a specific adaptive response by the cancer cells, which is then selectively targeted by a follow-on therapy. **We hypothesize that pre-treatment with androgen receptor (AR) agonists will drive cells into an adaptive response mechanism dependent on androgen, increasing efficacy of endocrine/CDK combination.** This adaptive response is subsequently targetable with AR antagonist (such as anti-androgen therapies or potentially DNA vaccine immunotherapy).



Clinical evidence supporting our approach: Recently, anti-androgen (enzalutamide) treatment in late-stage mBC in combination with endocrine therapy (exemestane) extended progression free survival (trial identifier: NCT02007512). When subgrouped by AR low versus AR high, anti-androgen had a longer median PFS compared with the control arm. The benefit disappeared in the AR low population, leading us to hypothesize that AR agonist pre-treatment in the AR-low population will increase AR-dependence as an adaptive response. Importantly, this adaptive response is targetable with anti-androgen. Thus, AR-agonist pre-treatment is a promising evolutionary steering approach in mBC using safe, clinically available treatments. Additionally, the AR-targeting agonist enobosarm has a FDA fast-track designation. *We hypothesize that AR-agonist pre-treatment increases AR-dependence in mBC patients, subsequently sensitizing response to endocrine therapies in combination with anti-androgen therapy.* To test this hypothesis, we propose these aims:

Aim 1: **Determine the evolution of estrogen and androgen dependence in breast cancer cell lines.** We consider four cell states within heterogeneous tumors: estrogen independent (Ei) or dependent (Ed) with androgen independent (Ai) or dependent (Ad). We hypothesize cell states will have differential growth rates, therapeutic response and population dynamics between cell lines. We will:

1a. Establish isogenic models of breast cancer possessing differential ER and AR status and test therapeutic response to AR agonists, aromatase inhibitors and anti-androgens.

1b. Define evolutionary dynamics of competing states of ER and AR dependence in isogenic cell lines upon treatment with AR agonists, aromatase inhibitors and anti-androgens.

1c. Develop, calibrate, and analyze a mathematical modeling framework of treatment-dependent cell state treatment-induced death rates using ordinary differential equations.

Aim 2: **Investigate the feasibility of an evolutionary double-bind sequence 1) AR agonists followed by 2) fulvestrant/aromatase inhibitors with anti-androgen therapy to prolong the emergence of resistance.** Despite clinical evidence that various AR agonists induce expression of AR and androgen dependence, the best follow up timing for aromatase inhibition and anti-androgens therapy remains unclear. In this aim, we investigate the feasibility of an evolutionary double-bind sequence. We will:

2a. Determine the role of androgen receptor activation and significance of AR agonist therapy in the treatment of metastatic breast cancer in immune deficient as well as immune competent *in vivo* settings.

2b. Investigate timing of AR agonists and fulvestrant/anti-androgen double-bind using *in silico* math models.

2c. Validate feasibility and beneficial effects of AR agonists administration prior standard of care combined with anti-androgens therapy in immune competent *in vivo* settings.

Aim 3: Unravel the role of spatial heterogeneity in mBC evolutionary double-bind therapy.

To better reflect the state of heterogeneity in clinical translation, we plan to extend the mathematical modeling of the previous aims to include spatially-explicit, heterogeneous cell populations. We will:

3a. Investigate the role of vascular-dependent drug perfusion on ER and AR dependence heterogeneity using an agent-based modeling framework.

3b. Extend the agent-based modeling framework to investigate the potential of alternative evolutionary double-bind treatments such as DNA androgen receptor vaccine (pTVG-AR, MVI-118).