

## Background and rationale

About 70 % of breast cancers (BCs) are estrogen receptor (ER) positive. In post-menopausal women estrogens, synthesized primarily by human aromatase (HA) enzyme, exert a pro-oncogenic effect by binding and activating ER $\alpha$ . Endocrine therapies against BC rely on modulation of ER $\alpha$  via selective estrogen receptor modulators (SERMs), or on estrogen deprivation via HA inhibitors. Despite their beneficial effects, all drugs in clinical use have severe side-effects and resistance issues, some appearing after few years of treatment under the evolutionary pressure of the therapies. Recently, selected tamoxifen metabolites, used in clinics as SERMs, were shown to allosterically (endoxifen) and competitively (norendoxifen) inhibit HA. Moreover, norendoxifen stereoisomers can also differently modulate ER $\alpha$ /ER $\beta$  activity. ER $\beta$  acts as tumor suppressor, inhibiting ER $\alpha$  mediated cell proliferation and growth in many cells.

## Hypothesis.

These recent findings opened new avenues to exploit HA allosteric modulation for therapeutic intervention against BC and demonstrated that a multi-targeted endocrine therapy, modulating the activity of several molecular targets associated to BC, is in principle possible even with low-molecular-weight molecules.

My research group has recently elucidated the molecular mechanism of the allosteric HA inhibition exerted by SERMs and has preliminary results on the mechanism of concerted competitive inhibition of HA and ER $\alpha$  by endoxifen/norendoxifen.

## Experimental Design

I propose to identify novel drug candidates via an integrated computational/experimental mechanistic-based approach capable of: (i) allosterically inhibiting HA; (ii) inhibiting HA, antagonizing ER $\alpha$  (and/or the polymorphic variants responsible of resistance and metastatic progression (mER $\alpha$ )), and/or agonizing ER $\beta$ . (iii) To identify off-targets proteins of novel drug candidates (from this project) and clinically used drugs to estimate their potential side-effects.

To this aim multi-target high-throughput virtual-screening experiments, relying on methods of increasing accuracy (docking, molecular dynamics and free energy calculations) will be complemented by the synthesis and *in vitro* assays on BC cell lines. Potential off-target proteins will be identified by similarity ensemble searching algorithms and a hierarchical inverse-docking approach.

## Expected Results

This project aims at establishing the molecular basis for novel therapies acting on the main players of ER+ BC. Its main outcomes will be: (i) novel allosteric inhibitors of HA; (ii) novel multi-target (with a triple/dual activity) inhibitors of HA, and modulators of ER $\alpha$  (and/or mER $\alpha$ ) and/or ER $\beta$ .

The design of these drugs is based on a detailed atomistic understanding of the molecular mechanism of BC's onset, of its resistance incurrence and of the mechanism of drug action on BC therapeutic targets.

## Impact on Cancer

The novel drug candidates identified by this project may be beneficial to overcome, reduce or delay resistance issues and side-effects of current endocrine therapies.

Namely, multi-target drugs, modulating selected molecular determinants of BC, may have the advantage of (i) circumventing the administration of several drugs, avoiding cumulative side-effects for patients, (ii) overcoming *de novo* or acquired resistance of currently employed drugs ; (iii) offering a therapeutic opportunity for BC subtypes refractory to endocrine therapies. They may (iv) help to elucidate the role of different BC molecular determinants; (v) be used to develop personalized and timely-tuned therapies to use in different BC subtypes and at different stages of BC progression.

## Abstract

*Circa il 70% dei casi di cancro al seno dipendono dall'attivazione del recettore agli estrogeni. Gli estrogeni sono sintetizzati dall'enzima aromatasi umana (HA) e svolgono il loro effetto pro-oncogenico legandosi e attivando il recettore agli estrogeni di tipo alfa.*

*Le terapie impiegate si basano sulla modulazione del recettore agli estrogeni con l'uso di modulatori selettivi per questo recettore (SERM) o sulla inibizione della produzione di estrogeni attraverso l'inibizione dell'aromatasi umana. I farmaci in uso clinico sono caratterizzati da molti effetti collaterali e problemi di resistenza. Recentemente è stato dimostrato che alcuni metaboliti del tamoxifen, usato come SERM, possono inibire l'enzima aromatasi umana e modulare in modo diverso il recettore agli estrogeni di tipo alfa o beta.*

*In questo progetto seguendo un protocollo computazionale basato su metodi computazionali di accuratezza crescente (docking simulazioni di dinamica molecolare e metodi di free energy) identificheremo nuovi farmaci che inibiranno sia il recettore agli estrogeni di tipo alfa che l'enzima aromatasi. Questi farmaci verranno poi sintetizzati dall'Università di Bologna (Prof. S Gobbi) e testati in vitro su linee cellulari del cancro al seno (Dr. N Zaffaroni Istituto Nazionale dei tumori Milano).*