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Role of androgen receptors in breast cancer

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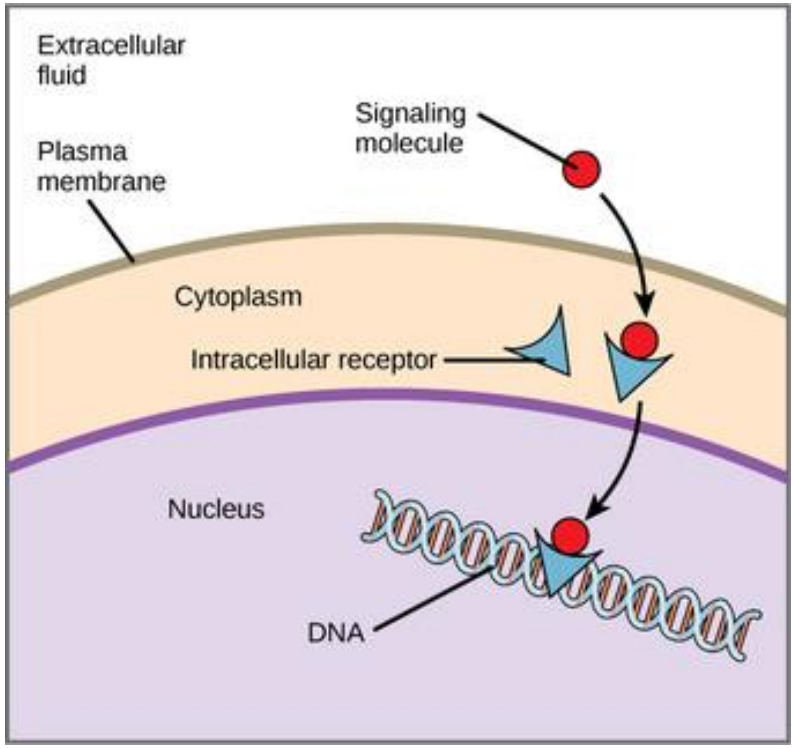
Androgen Receptor (AR)

- a memeber of the **steroid receptor family** that includes estrogen and progesterone receptors (ER and PgR rispectively)

- is predominantly localized to the cytoplasm complexed with molecular chaperones. Upon ligand binding, it translocates to the nucleus, directly associates with DNA regulating targeted genes transcription→ it is a **ligand-dependent transcription factor (TF)**



Genes targeted by AR

- Androgen signaling is critical for:

1. **development and maintenance of male sexual characteristics**
2. **female physiology and reproduction**

- Androgen signaling has been linked with many diseases:

1. Diabetes
2. Metabolic syndrome
3. Alzheimer's disease
4. **Prostate cancer**
5. **Breast cancer (BC)**

- A transcriptional network for AR co-expressed genes has been identified in BC→ it is significantly enriched with **cell cycle and metabolic genes** *(Naderi, Exp Cell Res 2015)*

- An **Androgen Responsive Gene Database (ARGDB)** has been built to provide integrated knowledge on androgen controlled genes

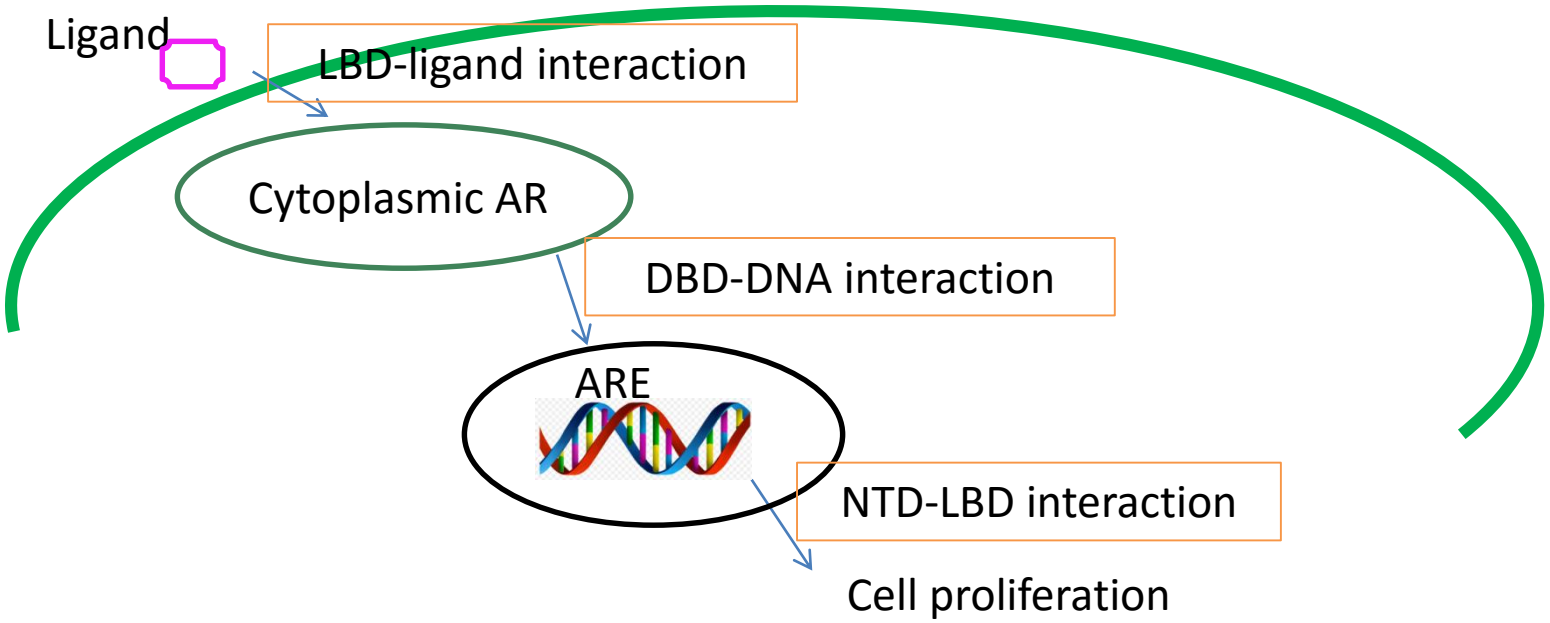
AR signaling

➢ AR structure consists of units, called "domains", that are functionally active and responsible for AR primary mechanism of action:

TRANSCRIPTIONAL/ GENOMIC MODE OF ACTION

1. Ligand binding leads to a rearrangement in LBD, inducing AR translocation to the nucleus
2. DBD allows recognition of DNA androgen response elements (AREs) and dimerization of receptor on DNA
3. NTD–LBD interaction induces AR transcriptional-related activity

(Higa, Int J Breast Cancer 2013; Kumar, Endocr Rev 2012)

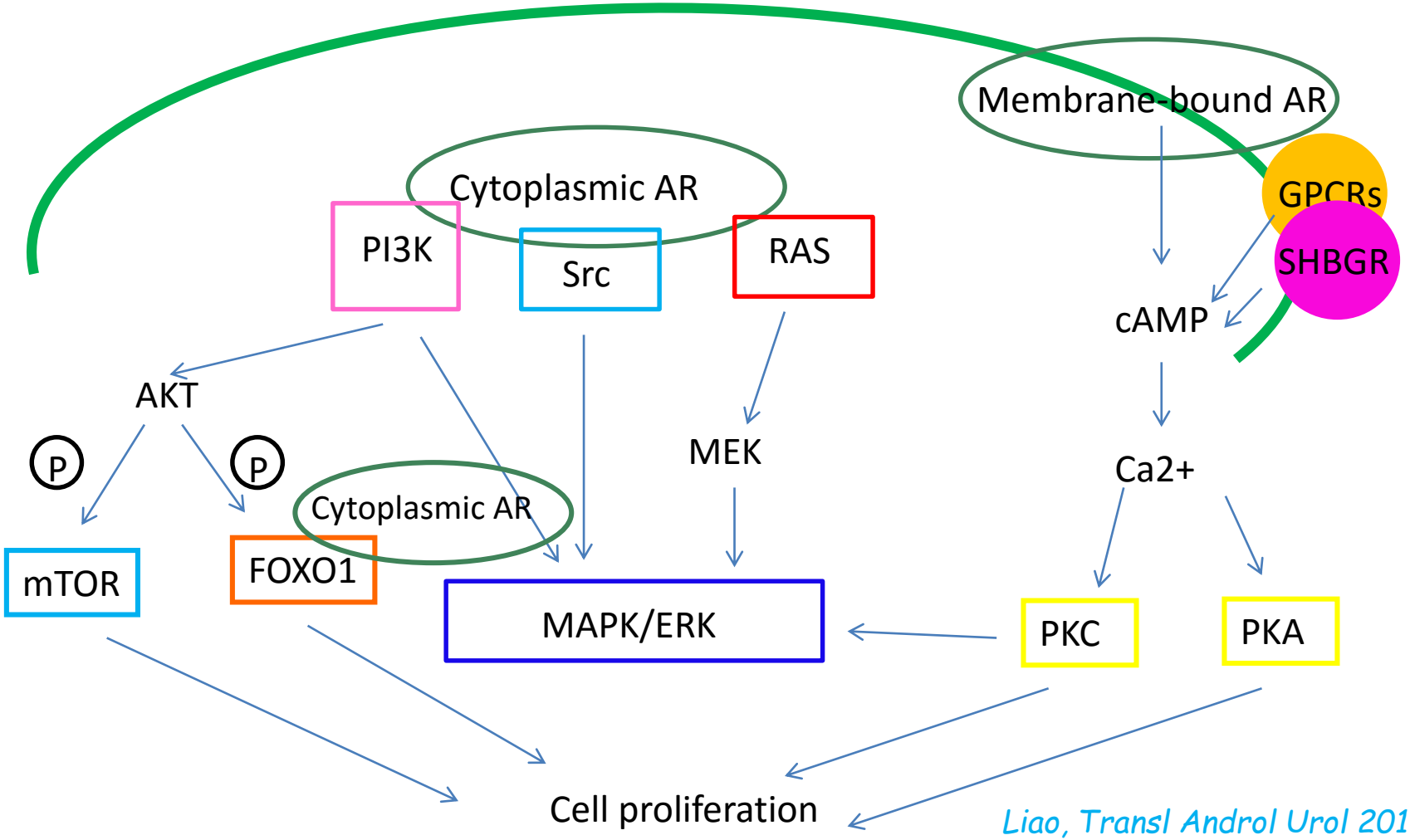


AR signaling

➢AR has also a second mode of action, independent of its interaction with DNA but involving **conventional elements of signal transduction pathways** and different **membrane-bound receptors:**

NON-TRANSCRIPTIONAL/NON-GENOMIC MODE OF ACTION

that may occur in an **ERK-dependent or ERK independent manner**

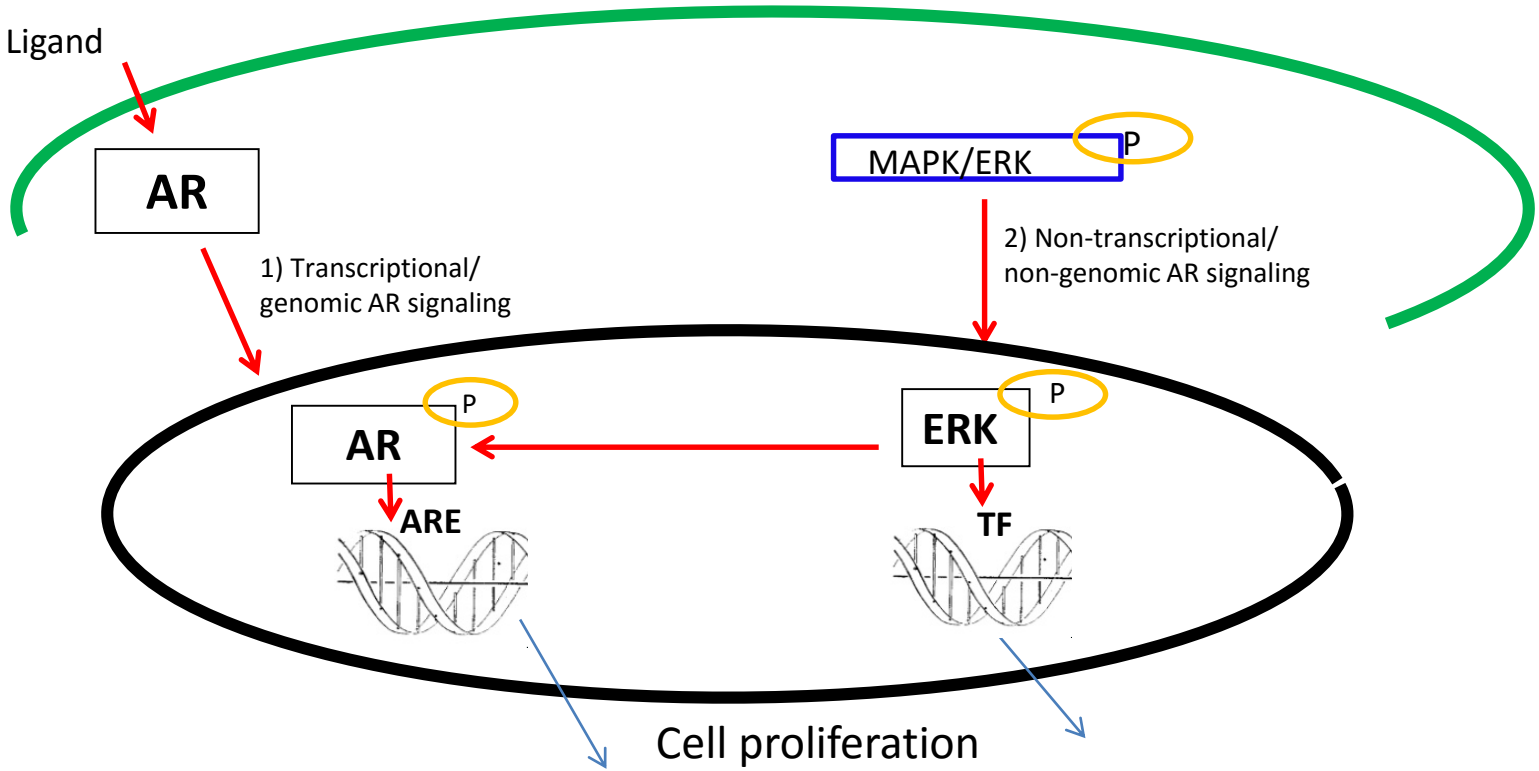


Liao, Transl Androl Urol 2013

AR signaling

➢A **cross talk** exists between these two pathways because ERK directly phosphorylates AR and its co-regulators *(Liao, Transl Androl Urol 2013)* →

amplified response to androgenic stimulation



resistance to anti-androgenic therapy

AR genomic alterations

➢Genomic functional alterations of AR affecting its activity have been identified in BC:

1. constitutively active ligand-independent AR splice variants: **AR-Vs** *(Hickey, Oncotarget 2015)*

2. **CAG polymorphic repeat lengths** in AR N-terminal domain *(Cogliati , Int J Biol Markers 2015; Lee, Cancer Biomark 2015)*

➢AR genomic **alterations potentially influence BC prognosis and response to AR target treatment**

Analysis of DNA released by tumor cells (**circulating DNA**) could be useful in monitoring **cancer clone dynamics and genomic causes of treatment resistance**

AR prevalence

- AR **is very frequently expressed in BC: up to 90% of primary tumors and 75% of metastatic BC** (Lim, Endocr Relat Cancer 2016)

- The frequency of AR expression varies between breast cancer subtypes with **ER-positive cancers more likely to be AR-positive than ER-negative**

- **AR status is highly conserved during tumor progression** (Grogg, BMC Cancer 2015; D'Amato, Mol Cancer Res. 2016) **OTHERWISE discordant AR status** from the primary tumor to recurrent site has been observed in **few cases** (Grogg, BMC Cancer 2015)

Re-testing of AR status on recurrence in case of primary AR-negative tumors is suggested
The same approach should be discussed in case of AR positive primary tumors

AR prognostic role

- There is an increasing evidence that **prognosis of women with AR-positive BC is typically context dependent →AR action depends on ER expression** *(Lim E, Endocr Relat Cancer 2016)*

- Many data confirm AR expression as an independent **prognostic factor of good prognosis in the context of ER-positive disease** *(Wang, Oncotarget 2016; Lim,Endocr Relat Cancer 2016; Aleskandarany, Breast Cancer Res Treat. 2016)*→ like ER, AR positivity may be indicative of a more well-differentiated status

WHEREAS

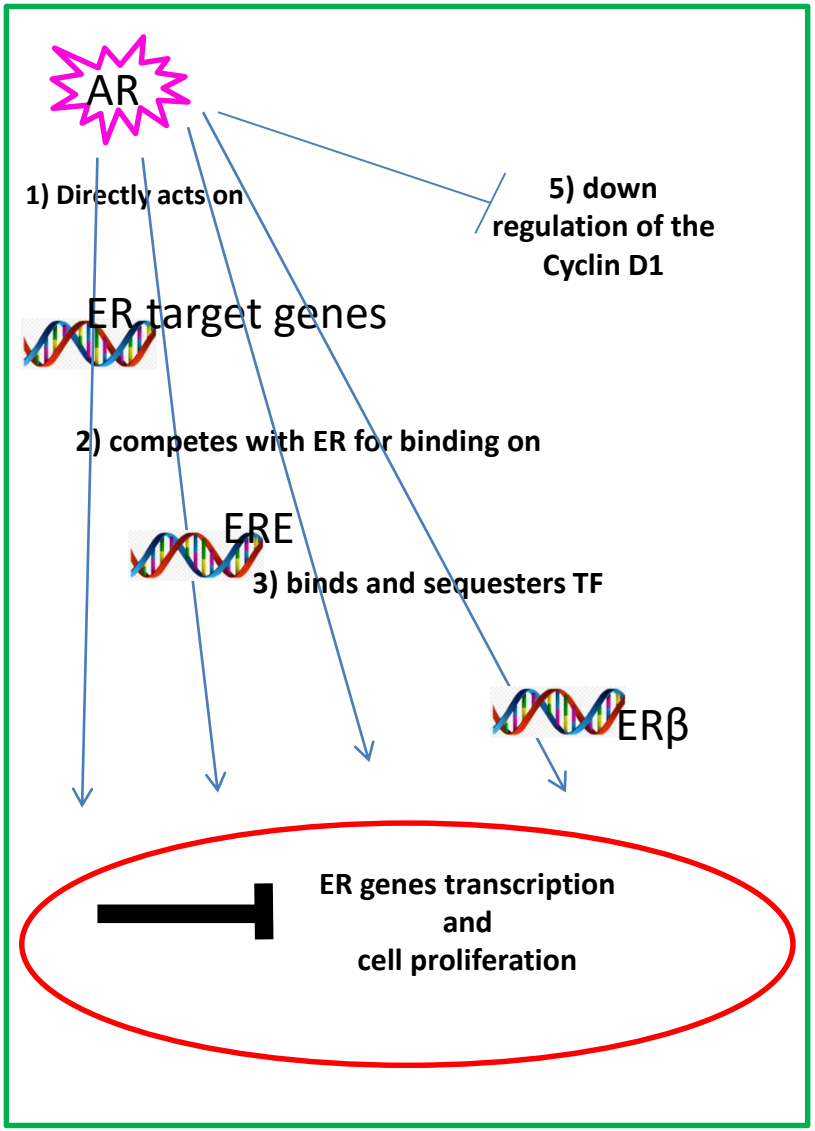
AR favorable prognostic significance is not uniform in the context of TNBC *(Rampurwala, Clin Adv Hematol Oncol 2016; Wang, Oncotarget 2016)*→ TNBC is inherently an heterogeneous disease

Evaluation of AR functional role in different BC subtypes may be useful to elucidate its prognostic implications

AR role in ER-positive BC

Clinical and pre-clinical data support evidence of **suppressive role of AR signaling in ER-positive BC**

(Lim E, Endocr Relat Cancer 2016)



Hypotheses

(1) AR directly inhibits ER target genes
(2) AR competes with ER in binding on estrogen response elements (EREs), avoiding ER-dependent gene transcription
(3) AR binds and sequesters TFs no longer available for transcription
(4) AR upregulates ERβ receptors
(5) AR downregulates cyclin D1 gene expression inducing apoptosis

AR role in ER-positive BC

Despite these data, AR role in ER-positive BC cannot be categorically defined suppressive

Conflicting data suggest a proliferative or protective AR effect on ER-positive/AR-positive BC cells

(D'Amato, Mol Cancer Res. 2016)

Possible explanations:

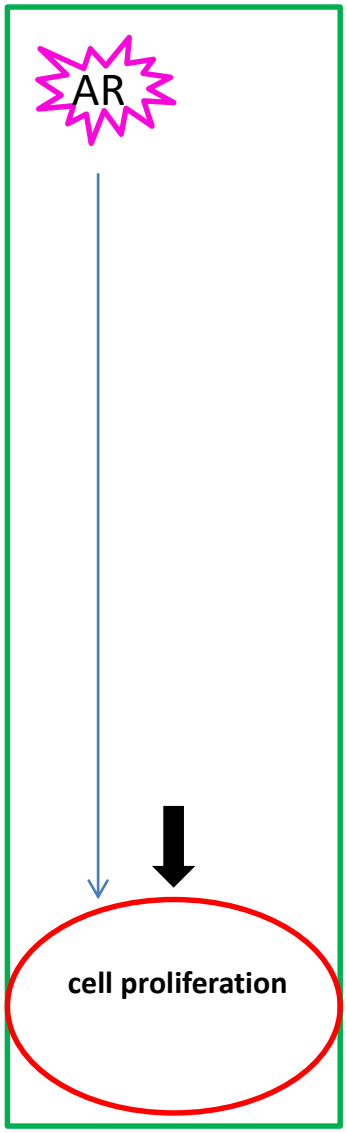
- androgen metabolites have estrogenic effects
- androgens bind to receptors other than AR
- AR directly induces cell proliferation
- As recently demonstrated, nuclear AR is critical for ER function → AR supports maximum ER genomic binding and activity

These recent data support the notion that **nuclear interplay of AR and ER (AR:ER ratio), reflects AR prolifertive otcome** depending on their relative expression levels, ligands levels, and shared co-factors levels *(Lim, Endocr Relat Cancer 2016)*

AR role in ER-negative BC

In ER-negative BC, AR can exert **both oncogenic and tumour suppressive effects**, even in the same model

(Hickey Mol Endocrinol 2012;
Lim. Current Breast Cancer Reports 2014;
Chia, Curr Oncol Rep 2015;
Nahlel Future Oncology 2008)



BUT focusing on **luminal AR (LAR) TNBC subtype**, showing gene expression signature enriched in hormonally regulated pathways including steroid synthesis (high AR expression)

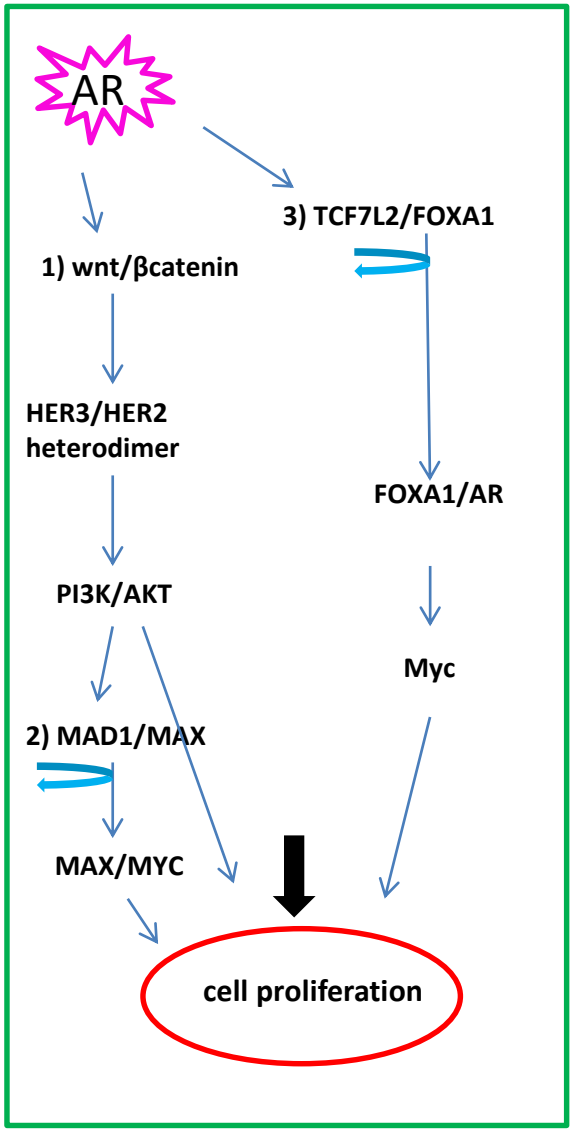
(Lehmann , J Clin Invest. 2011)

AR is an oncogenic driver of tumor growth

(Robinson, et al. 2011; Chia, Curr Oncol Rep. 2015)

AR role in ER-negative/HER2-positive BC

In **HER2-positive BC, in the absence of ER expression**, there is **strong evidence of a proliferative role of AR signaling**



Preclinical data suggest :

1. AR directly induces WNT7B expression activating **WNT/β- CATENIN pathway** with subsequent induction of HER3 gene transcription →HER3 forms heterodimers with HER2 modulating PI3K/AKT pathway in a cell proliferative direction

2. Through PI3K/AKT pathway, AR phosphorylates **MAD1** (competitor of MYC for MAX interaction) → MAD1 dissociates from MAX→ MAX/MYC heterodimerization with enhanced MYC activity

3. AR induces dissociation of transcription factor 7-like 2 **(TCF7L2)** from **FOXA1*** at AR binding site → inducing AR targeted genes expression, including MYC

*FOXA1 is highly expressed in HER2 positive BC. TCF7L2 physical interaction with FOXA1 mediates transcriptional repression of AR targeted genes

Targeting AR

- It clearly appears that **AR plays a key role on BC cell growth BUT conflicting data suggest its proliferative or protective effect even in the same BC subtype**

- **AR proliferative outcome is not fully defined by AR expression alone in different BC subtypes → it depends on AR/ER interplay and on AR interaction with elements of pathways contributing to BC development**

- **AR represents an important therapeutic target: AR-targeting treatment** potentially represents a new target therapy in **ER-negative BC (TNBC as HER2-positive BC)** and an **additional hormonal treatment option in ER-positive tumors**

The two major current challenges:

1) To identify **in what way AR has to be targeted (activated or blocked) in each BC patient for obtaining an antitumor effect**, basing on biological and molecular tumor characteristics, preclinical data of drug effect on BC cell growth, available phase I data of drug activity

2) To identify the **optimal AR-targeting treatment setting and combination/sequencing with other conventional drugs**

Targeting AR

- Many **AR-targeting drugs are currently under evaluation** in Phase I and II clinical trials
- AR-targeting drugs include **AR activators/agonists and inhibitors/antagonists**
- Conflicting data regarding AR role and drug effects on breast cancer cells growth suggest **evaluation of both AR agonists and antagonists activity across different BC subtypes, even regardless AR expression**
- It is possible to classify AR-targeting drugs according to their mechanism of action

Targeting AR

A) ANDROGENS

1. DHEA:

- androgen precursor with limited or absent virilizing effects
- Preclinical data → inhibitory effect on both ER-positive and ER-negative BC cell lines

2. 4-OH-testosterone:

- Anabolic steroid with aromatase inhibiting activity
- Preclinical studies → anti-proliferative activity in both ER-positive and TNBC cell lines

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Dehydroepiandrosterone (DHEA) + romatase inhibitor (AI)*	II	Endocrine responsive/HER2neg/ARpos and TN/ARpos mBC	NCT02000375	/
4-OH-testosterone (CR1447) with transdermal administration**	II	Endocrine responsive/HER2neg/anyAR and TN/ARpos locally advanced or mBC	NCT02067741	/

- *DHEA-AI combination → for preventing DHEA conversion into estrogens and maximizing the amount of androgens available
- ** Transdermal application → for obtaining continuously 4-OHT release and omitting a first pass effect.

Targeting AR

B) SARMs (selective AR modulators)

1) Enoborsam (GTx-024, ostarine)

- SARM without cutaneous androgenic activity. It's not convertible into estrogenic metabolites

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Enoborsam (GTx-024)	II	ERpos/anyAR mBC	NCT01616758	AR subset analysis: ▪primary endpoint has been achieved, with 35% clinical benefit rate (CBR) at 6 months. ▪good treatment tolerability *(Overmoyer, Cancer Res 2016)*
Enoborsam (GTx-024)	II	ERpos/ARpos locally advanced or mBC	NCT02463032	/
Enoborsam (GTx-024)	window	ERpos/ARpos early untreated BC	EMERALD	/

Targeting AR

C) ENZYME INHIBITORS

1) Abiraterone Acetate (AA)

➢androgen synthesis inhibitor through inhibition of the two enzyme activity (17alpha-hydroxylase and 17,20-lyase) of CYP17A1, a member of the cytochrome P450 superfamily involved in sex hormones synthesis → reduction in serum levels of estrogens and androgens

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Abiraterone Acetate	Phase I/II	ERpos/any AR or ERneg/ARpos mBC	NCT00755885	**ER-positive group**: CBR of 21% at 24 weeks with good tolerability (*Ng, ESMO 2012, Abstract 325PD*)
Abiraterone Acetate + Prednisone With or Without Exemestane	II	ERpos mBC	NCT01381874	No improvement of PFS adding AA to exemestane* (*O'Shaughnessy, JCO 2016*)

* AA-induced progesterone increase may have contributed to this lack of clinical activity

Targeting AR

C) ENZYME INHIBITORS

2) Orteronel

- inhibitor of 17,20-lyase enzyme activity of CYP17A1 → reduction of androgens and estrogens levels

- Phase I study in ER-positive mBC → well tolerated with evidence of clinical benefit *(Murtuza, JCO 2014 suppl abstr 538, NCT01808040)*

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Orteronel (TAK-700)	II	Endocrine responsive/ARpos or TN/ARpos mBC	NCT01990209	/

Targeting AR

C) ENZYME INHIBITORS

3) VT-464

➢second-generation small molecule inhibitor of 17,20-lyase enzyme activity of CYP17A1 → → reduction of androgens and estrogens levels

Agent	Phase	Population	References/ Clinicaltrils.gov	Available data
VT-464	I/II	PI: ERpos/HER2neg or TN mBC PII: ERpos/HER2neg/ARpos or TN/AR pos mBC	NCT02144051	/

Targeting AR

C) ENZYME INHIBITORS

3) Irosustat (STX64)

➢Inhibitor of steroid sulfatase (STS) that catalyzes the conversion of sulfated steroid precursors to the free steroids → preventing formation of steroids with potent estrogenic properties (DHEA and estrone)

➢Two phase I studies → good tolerability and evidence of clinical activity in BC patents *(Stanway, Clin cancer Res 2006; Coombes, Breast Cancer Res Treat. 2013)*

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Irosustat + AI*	II	ERpos Locally Advanced or mBC	NCT01785992	/
Irosustat	window	ERpos early BC	NCT01662726	/

* AI is continued beyond disease progression

Targeting AR

D) AR ANTAGONISTS

1) Bicalutamide

- non-steroidal anti-androgen that competitively inhibits the binding of androgens with AR

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Bicalutamide	II	ERneg/PgRneg/HERany /ARpos mBC	NCT00468715	26 evaluable pts: 6-months CBR of 19%, well tolerated treatment *(Gucalp, Clinical Cancer Research 2013)*
Bicalutamide + Palbociclib *	I/II	PI: ERany/ARpos mBC PII: TN/ARpos mBC	NCT02605486	/

•Palbociclib is an inhibitor of CDK (cyclin-dependent kinases) 4 and -6, members of the cell-cycle regulatory machinery complexing with their cyclin partners allowing for cell cycle progress
•preclinical evidence of efficacy of CDK4/6 inhibitors in LAR TNBC cell lines

Targeting AR

D) AR ANTAGONISTS

2) Enzalutamide

➢Another non-steroidal anti-androgen that competitively inhibits androgen binding and even prevents AR nuclear translocation and interaction with chromatin

➢In-vitro and in-vivo data show its capacity of blocking androgen and estrogen –mediated growth in ER-positive and ER-negative/AR-positive BC cells by inhibiting AR nuclear localization

➢Effecively synergizes with antiandrogens in ER-positive/AR-positive BC cells

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
Enzalutamide alone or enzalutamide + AI or enzalutamide + fulvestrant	I	Any mBC	NCT01597193	/
Enzalutamide + exemestane	II	Endocrine responsive mBC	NCT02007512	/
Enzalutamide + trastuzumab	II	ARpos/HER2pos locally advanced or mBC	NCT02091960	/
Enzalutamide + exemestane or Enzalutamide alone	window	Early ERpos or TN/ARpos BC	NCT02676986	/

Targeting AR

D) AR ANTAGONISTS

Agent	Phase	Population	References/ Clinicaltrils.gov	Available data
Enzalutamide + taselisib*	I/II	TN/ARpos mBC	NCT02457910	/

- Taselisib is a PI3K inhibitor
- AR-positive TNBC have a higher frequency of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha gene (PIK3CA) mutations compared with AR-negative
- Bicalutamide combined with a pan–phosphoinositide 3-kinase (PI3K) inhibitor has shown additive effects

Targeting AR

D) AR ANTAGONISTS

3) AZD5312 (ISIS-ARRx)

➢antisense nucleotide targeting AR mRNA physically obstructing the mRNA translation machinery

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
AZD5312	II	ARpos solid tumors	NCT02144051	/

4) Ganetespib (STA-9090)

➢a heat shock protein inhibitor that, interacting with the molecular chaperone HSP90, causes AR inactivation

Agent	Phase	Population	References/ Clinicaltrials.gov	Available data
STA-9090	II	AnyER/any HER2 mBC	NCT01273896	/
STA-9090	II	ERpos or HER2pos orTN mBC	NCT01677455	/

Future prospectives

1) AR antagonists after neoadjuvant treatment in TNBC patients

- AR-positive tumors have lower pathological complete response (pCR) rate compared to AR-negative tumors →

AR expression predicts chemoresistence independently from hormonal receptor status

(Loibl, Breast Cancer Treat 2011, Rampurwala, Clin Adv Hematol Oncol 2016, Asano, BJC 2016)

- The **strongest association between pCR and outcome** is found in pts with TNBC

(Cortazar P, Lancet 2014)

Targeting AR offers a biologically promising strategy in **AR-positive/TNBC with high risk of relapse after surgery due to low pCR rate**

We can hypothesize two different therapeutical strategies:

1) **Combination of anti-androgen compounds with chemotherapy in neoadjuvant setting** for obtaining higher pCR rate and better outcome

2) **Anti-androgen compounds** as **additional treatment in case of unachieved pCR after neoadjuvant chemotherapy** for eliminating resistant clones

Future prospectives

2) AR antagonists in endocrine-resistant ER-positive BC patients

Androgen signaling and AR are involved in resistence to ER directed endocrine therapies and this may be due to tumor adaptation to androgen dependence

- **AR overexpression in BC cell lines resulte in resistance to tamoxifen (TAM) and AI in vitro and in vivo** *(D'Amato, Mol Cancer Res. 2016)*

- Recent data suggest that **high ratio of AR:ER indicates increased risk for failure while on TAM** *(Cochrane, Breast Cancer Res 2014)*

Future prospectives

2) AR antagonists in endocrine resistant ER-positive BC patients

➢It has been recently demostrated that **Enzalutamide**

1. **inhibits both androgen and estrogen induced growth of ER-positive/AR-positive cells** in vitro and in vivo by inhibiting AR nuclear localization
2. effectively **synergizes with anti-estrogens** by inhibiting AR as well as indirectly ER
3. **inhibits TAM-resistant tumor growth and is effective in reducing growth of metastatic disease**

(D'Amato, Mol Cancer Res. 2016)

Enzalutamide may be used
in combination with traditional endocrine therapies, particularly in case of high AR:ER ratio
or as a single agent in tumors resistent to traditional endocrine therapies

Conclusions

- **AR** is expressed in the majority of BC and plays a key role in BC cell growth → **it represents an important therapeutic target**

- **AR-targeting treatment** potentially represents a new target therapy in **ER-negative BC (TNBC as HER2-positive BC)** and an additional hormonal treatment option in **ER-positive tumors**

- **AR proliferative outcome is not fully defined by AR expression alone in different BC subtypes → it depends on AR/ER interplay and on AR interaction with elements of pathways contributing to BC development.** The challenge is to identify **in what way AR has to be targeted (activated or blocked) in each BC patient for obtaining an antitumor effect**

- Conflicting data regarding AR role and drug effects on breast cancer cells growth suggest **evaluation of both AR agonists and antagonists activity across different BC subtypes, even regardless AR expression.** A subsequent deep evaluation of ER/AR/HER2 expression, AR/ER nuclear ratio, pathways contributing to BC development and drug mechanism of action could subsequently allow us to identify biological features (to be validated in prospective studies) for optimizing AR targeting therapy

Conclusions

- **AR-targeting compounds** are currently studied as **advanced lines of treatment** to evaluate their safety and activity

- Future studies should evaluate the optimal **sequencing of AR-targeting treatment with other conventional anti-estrogen drugs in ER-positive BC patients and AR-targeting treatment use as first-line therapy in selected ER-negative and HER2-positive BC patients**

- Use of AR-targeting treatment **in neoadjuvat setting** could identify **predictive biomarkers** of single agents and drug combinations. They should be later used in **adjuvant treatment decision making**

- Currently, there are clinical and preclinical basis for evaluating **AR antagonists in AR-positive/TNBC patients with residual disease after neoadjuvat chemotherapy** and **enzalutamide in combination with traditional endocrine therapies or as a single agent in endocrine-resistant tumors**

- Analysis of AR-targeting treatment activity should take into consideration the **cross-talk existing between genomic and non-genomic AR mode of action and the identification of AR genomic alterations** that potentially influence final treatment results

I would like to thank
Prof Amadori
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to this congress

And last but not least,
I would like to thank all of my colleagues, especially those of the breast group, for supporting me in my research

Thank you for your attention