Fine-Tuning Adjuvant Endocrine Therapy for Early-Stage Breast Cancer: An Expert Consensus on Open Issues for Future Research

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Running title: An Expert Consensus on Open Issues for Future Research

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Disclosure/competing interest: Prof. Gelber declares that his institutions receive grant support for his salary from Roche**,** AstraZeneca, Merck, Novartis.

Prof. Viale has received grants/research supports from Roche/Genentech, Ventana Medical Systems, Dako/Agilent Technologies, and honoraria or consultation fees from

Ventana, Dako/Agilent, Roche, MSD Oncology, AstraZeneca, Daiichi Sankyo, Pfizer, and Eli Lilly.

All other authors declare no conflicts of interest.

Abstract.

After decades of research, improving the efficacy of adjuvant endocrine therapy (ET) for early-stage breast cancer (BC) becomes increasingly difficult. Beyond technological breakthroughs and the availability of new classes of drugs, further improvement of adjuvant ET will require applying a rigorous research approach in poorly investigated areas. We critically discuss some key principles that should inform future research to improve ET efficacy, including identifying specific subgroups of patients who can benefit from escalating or de-escalating approaches, optimizing available and new treatment strategies for different clinical contexts, and dissecting the direct and indirect biological effects of therapeutic interventions. Four main issues regarding adjuvant ET were identified as relevant areas where a better application of such principles can provide positive results in the near future: i) tailoring the optimal duration of adjuvant ET; ii) optimizing ovarian function suppression for pre-menopausal women; iii) dissecting the biological effects of estrogen receptor manipulation; and iv) refining the selection of patients to candidate for treatments escalation.

Introduction.

Endocrine therapy (ET) is the most effective adjuvant treatment for patients with early-stage hormone-receptor-positive (HR+) breast cancer (BC).[1]

There is now a wide range of anti-estrogen drugs whose efficacy is well established in adjuvant or neoadjuvant setting, including the selective estrogen receptor modulator (SERM) tamoxifen, selective estrogen receptor downregulators (SERDs), such as fulvestrant, the aromatase inhibitors (AIs) letrozole, anastrozole and exemestane, which block the conversion of androgens into estrogens, and the gonadotropin-releasing hormone agonists (GnRHs) goserelin, leuprorelin and triptorelin, used to obtain ovarian function suppression (OFS) in premenopausal women.[1]

The amount of benefit provided by such therapies is clinically meaningful. Indeed, in postmenopausal women, 5 years of adjuvant treatment with tamoxifen reduces the relative risk of recurrence as compared with no ET by about half during the first 5 years after surgery and one-third during the subsequent 5 years and reduces the breast cancer mortality rate by about 30%. Five years of adjuvant AIs further reduce the relative risk of recurrence by 30% as compared to tamoxifen, as well as the 10-year breast cancer mortality rates by about 15%.[1] Though these proportional reductions in risk of recurrence are substantially independent of prognostic factors such as nodal status, tumor grade and size, these factors substantially affect the absolute risk with no ET, and hence also affect the absolute reduction in that risk obtained by adjuvant ET.[1]

Since so much progress has been achieved during previous decades, a further improvement of adjuvant ET requires the application of principles such as identifying specific subgroups of patients who can benefit from escalating or de-escalating approaches, optimizing available and new treatment strategies for different clinical

contexts, and dissecting the direct and indirect biological effects of therapeutic interventions.[2,3]

There are several issues regarding adjuvant ET for early-stage BC, where one or more of above-mentioned principles have remained poorly applied. Four particularly relevant examples reviewed in this paper are: i) tailoring the optimal duration of adjuvant ET; ii) optimizing use of ovarian function suppression (OFS) for pre-menopausal women; iii) dissecting the biological effects of Estrogen Receptor (ER) manipulation; and iv) refining the selection of patients to candidate for treatments escalation or descalation.

Tailoring the optimal duration of adjuvant ET.

Despite many randomized clinical trials (RCTs) conducted in the last decades, uncertainty still exists regarding the optimal duration of adjuvant ET.[2,4] Issues requiring further studies are the identification of patients who can derive a clinically meaningful benefit from extended versus standard ET; the optimal duration of such treatment extension; and the management of specific subgroups of patients poorly represented in RCTs.

Results of several RCTs showed that ET extended to 10 years significantly improves the disease-free survival (DFS) as compared with standard 5 years of treatment (**Table 1**). Final positive results were reported in the aTTom and ATLAS trials comparing 10 versus 5 years of tamoxifen, in the MA17 trial comparing 5 years of AI versus placebo after the first 5 years of therapy with tamoxifen, and in the NSABP-42 trial testing 5 years of extended AI following AI given upfront for the first 5 years or switched after ≤3 years of tamoxifen. [5-8]

In all of these trials, the absolute DFS-benefit of extended ET was limited to 3-5 percentage points, but was burdened by an increased risk of adverse events, including

up to 5% absolute higher risk of bone fractures.[5-8] Furthermore, the amount of benefit provided by extended adjuvant ET with AIs depends on the type of ET received in the first 5 years of treatment, being smaller for women who received AIs for at least part of their initial ET as compared with those treated with only tamoxifen.[9]

However, it has been robustly shown that the risk of relapse of HR+BC persists for decades after the diagnosis, as well as that adjuvant ET, differently from chemotherapy, is characterized by a long-lasting carry-over effect.
For these reasons, a follow-up substantially longer than reported in the available trials would be necessary to reliably capture the absolute benefit of the extended ET.[10]
In the ATLAS trial, 77% of patients have been followed up for 15 years after diagnosis, and it is therefore the only trial with a follow-up long enough to address this issue.[6] Notably, a progressive increase of benefit from extended ET in the second decade after diagnosis has been documented in the ATLAS trial.[6]

Furthermore, specific subgroups of patients may derive substantially higher absolute benefit than others. A comprehensive evaluation of baseline clinico-pathological variables (i.e., age, tumor size, grade and lymph node status) through the prognostic model called CTS5, is useful to estimate distant-recurrence (DR) risk after 5 years of ET.[11] CTS5 may help to stratify women into different risk categories, the lowest of which has only a 3.6% risk of DR in years 5–10 and hence can be spared from extended ET. Unfortunately, since the CTS5 predictive value for benefit from extended ET has not been demonstrated, this model does not help to identify patients in higher risk categories whose disease warrants consideration of extended ET.[11]

A refinement of the predictive factors to inform the selection of patients who are candidates for extended ET is thus urgently needed.
Biological factors that underpin the emergence of late recurrence are multiple and complex.[12] Some evidence showed that those patients with higher tumor ER levels

and without recurrence after 5 years of ET, have a higher subsequent risk of recurrence than those with low ER levels.[13]

Many RNA-based molecular signatures capturing tumor biology are available, but to date only their prognostic value has been retrospectively demonstrated in this context.[14] The only two signatures with proven predictive value for benefit of extended ET are the HOXB13/IL17BR (H/I) ratio and 70-gene MammaPrint (MP) test. HOXB13 and IL17BR are two genes associated with activation of the estrogen receptor(ER) signaling pathway.[15] Retrospective analyses showed that the H/I-ratio predicted benefit from different types of extended adjuvant ET, such as 10 years of tamoxifen in the aTTom trial, 5 years of tamoxifen followed by additional 5 years of AI in the MA17 trial, and 10 years of AI in the NSABP-42 trial.[16-18] In all these studies, patients with low H/I-ratio had no benefit from extended ET, whereas those with high H/I-ratio had a statistically significant absolute reduction of 3.6% of the DR risk in the NSABP-42 trial, and of 10% and 16% of the risk of recurrence in the aTTom and MA17 trials, respectively.[16-18]

The 70-gene MammaPrint (MP) assay predicts the risk of DR and classifies HR+ early-stage BC as low- or high-risk. A retrospective analysis of the NSABP-42 trial, showed that patients with MP low-risk tumors had a statistically significant absolute reduction of 4% of the DR risk with extended ET, while a smaller and not significant benefit was observed for MP high-risk tumors.[19]

Although these results are promising, a prospective validation of both H/I ratio and MP is still needed for acceptance of their predictive value, considering the not negligible risk of bias of such type of retrospective analyses, including the attrition bias due to limited number of tumor samples available for the analyses.[16-18] Furthermore, since most analyzed patients had 3 or less positive lymph nodes, evidence to support the predictive value of the H/I-ratio and MP in patients with more advanced stage of disease is very limited.[16-18]

The optimal duration of extended ET is a further issue that should be addressed.

To date, three RCTs (i.e., SALSA, IDEAL and DATA trial) compared a "full-extended" adjuvant ET (i.e., more than 7.5 years of treatment) versus a "limited-extended" ET (i.e., more than 5 but less than 7.5 years of treatment overall).[20-22] Results for the intention-to-treat (ITT) populations of all these trials, showed no significant differences between treatment arms. [20-22]

Furthermore, a retrospective analysis of the IDEAL trial showed that the CTS5 was not predictive of benefit from the full- versus limited-extended adjuvant ET, since there was no risk category in which the treatment allocation had a significant effect on the risk of DR.[23]

However, we recently performed a meta-analysis of the SALSA, IDEAL and DATA trials, having enough statistical power to show a significant interaction between nodal status and efficacy of the full- versus limited-extended ET. No benefit was observed in patients with node-negative disease, whereas those with node-positive disease had significantly improved DFS with the full- as compared with the limited-extended ET.[24]

Notably, retrospective evidence suggests that the H/I ratio and MammaPrint assays could help to establish the optimal duration of extended ET.[25,26]

In the IDEAL trial, patients with node-positive disease but low H/I-ratio had no benefit from extending ET up to 10 years as compared with 7.5 years, while patients with node-positive disease and high H/I ratio obtained a statistically significant and clinically meaningful absolute RFI-benefit of 11% when treated up to 10 years.[25]A similar analysis conducted on the IDEAL trial with the MP assay, showed that patients with MP low-risk tumors obtained a significant absolute RFI-benefit of 10% from the full- versus limited-extended ET, while patients with MP high-risk tumors did not derive benefit.[26]

Taken together, these data show that an "one-fits-all" approach for selecting the optimal duration of ET is not suitable, and raise the hypothesis that a tailored escalation/de-escalation strategy, ranging from standard 5 years to 7-8 years for the limited-extended, and up to 10 years for the full-extended ET, should be applied case- by-case based on the clinico-pathological and tumor molecular features of each patient

Variables dynamically collected during (neo)adjuvant ET could complement baseline clinico-pathological factors, to further personalize ET duration. As discussed below, detection of circulating tumor DNA at the end of the standard 5 years of ET, may help to identify those patients at higher risk of relapse who need to continue ET.[27-28]

It should also be considered that there are several patient subgroups, such as premenopausal women, patients with rare tumor histotype, or those exposed to new targeted therapies in the first years of adjuvant ET, for whom evidence of benefit from extended ET is lacking.

The large majority of patients enrolled in trials testing extended ET were post-menopausal women.[1] The only evidence available for premenopausal women comes from the aTTom and ATLAS trials, that confirmed an improved patients' outcome by extending therapy with tamoxifen for 10 years, and from the MA17 trial, suggesting benefit by switching to 5 years of AI after 5 years of tamoxifen in premenopausal patients who become postmenopausal during treatment.[5-7] Evidence is missing, however, on the efficacy of extended ET for pre-menopausal patients after 5 years of treatment with LH-RH plus AIs.

Although tumor histotype has been long recognized as significantly associated with the risk of relapse over time, histotype-disaggregated data from RCTs testing adjuvant extended ET have been rarely reported.[29] As compared with ductal carcinoma, lobular cancer has a very long-lasting risk of relapse.[29] Furthermore,

factors associated with the late-risk of relapse may be different in the two histotypes. We reported that the prognostic value of the CTS5 model might be significantly improved by integration of the Ki67 for lobular but not for ductal carcinoma.[30] On the other hand, tubular and cribriform carcinomas are characterized by higher endocrine sensitivity and better long-term prognosis than other histotypes.[31] The hypothesis that extended adjuvant ET may be particularly helpful for lobular cancer but not for the majority of tubular and cribriform tumors should be explored.

Finally, the recent results of the MonarchE and OlympiA trials, showing an improved outcome for patients treated respectively with 2 years of the CDK4/6 inhibitor abemaciclib or 1 year of the PARP inhibitor olaparib, further complicate defying the optimal duration of ET.[3,32] Indeed, how and if the administration of such new targeted therapies modifies the efficacy of extended ET is completely unknown.

Optimization of OFS for pre-menopausal women.

For a long time, clinical research on adjuvant ET was mainly focused on postmenopausal women, with a relevant delay in the optimization of adjuvant ET for premenopausal women.[1]

Only recently, evidence from RCTs showed OFS+AIs as the most effective adjuvant treatment in this patients' population.[33] While the benefit of such treatments as compared with tamoxifen in the ITT-population is limited, patients with adverse clinico-pathological risk factors (e.g., age <35 years, node-positive disease, tumors >2 cm or grade 3) and HER2-negative disease derived a clinically meaningful OS-benefit in the long-term follow-up analysis of the TEXT and SOFT trials.[34] Whether the selection of premenopausal patients candidate for OFS+AIs could be further improved by tumor molecular signatures is still an unaddressed issue. Recent evidence showed a significant interaction of the H/I ratio and the efficacy of OFS+AI versus tamoxifen in the SOFT trial.[35] Quite unexpectedly, however, the direction of

such interaction was the opposite of that observed in trials of extended adjuvant ET: the patients who benefited the most from OFS+AI, were those with a low rather than high H/I ratio.[35]

Consistently, a non-significant trend for larger breast cancer free interval (BCFI)-benefit from OFS+AI versus tamoxifen in patients with low or intermediate PAM50 ROR score (BCFI-HR: 0.5, 95%CI 0.3-0.8) as compared with those with high score (BCFI-HR 0.9, 95%CI 0.9-0.4; p-interaction:0.1) treated in the SOFT trial has been recently reported.

If such an unforeseen finding is a result of chance or relies on pathogenetic differences of HR+BC arising in pre- versus post-menopausal women, deserves further investigations.

Unfortunately, even when treated with OFS+AI for five years, the additional residual risk of distant recurrence in high-risk patients is not negligible, reaching about 20% at 12 years.[34] This highlights the urgent need to improve efficacy of adjuvant ET in such high-risk subgroup of patients. Optimizing OFS is one of the most crucial steps to achieve such objective. In current clinical practice, OFS is pursued through gonadotropin-releasing hormone (GnRH) analogs in most patients.[1] Some pharmacodynamic limitations, related to the agonistic mechanism of action of such drugs, may impair the induction of a successful OFS. A delay of 2 to 4 months from the start of treatment until downregulation of gonadotropins has been observed in some patients. Moreover, approximately 20% of women do not maintain a complete OFS with GnRH-analogs during treatment.[1,36] This can substantially impair the efficacy of adjuvant ET.

Three potential non-invasive therapeutic strategies, not mutually exclusive, are the most promising strategies to overcome a suboptimal OFS. The first option is the combination of chemotherapy and GnRH-analogs. The rate of chemotherapy-induced amenorrhea and its duration depend on patient's age and type of chemotherapy.[37]

When treated with anthracycline- and taxane-containing regimens, up to 60-80% of premenopausal women developed amenorrhea, and menses do not resume in up to 50% of women younger than 40 years and in the majority of women older than 40 years.[37] Such OFS effect of chemotherapy can synergize with that of GnRH-analogs. Indeed, during the first year of treatment with OFS+AI in the SOFT trial, 17% of patients did not achieve complete suppression of estradiol (E2) levels, and no prior exposure to chemotherapy was one of the few factors found significantly associated to unsuccessful OFS.[38] Furthermore, some evidence showed that adjuvant chemotherapy can improve outcome of premenopausal women with HR+BC through a dual mechanism of action: a direct cytotoxic effect and an indirect endocrine effect secondary to chemotherapy-induced OFS. This latter hypothesis is supported by many retrospective analyses of RCTs testing adjuvant chemotherapy, showing larger RFS-benefit in premenopausal women who achieved amenorrhea as compared with those who did not.[37] The recent results of the RxPONDER RCT, testing the efficacy of adjuvant chemotherapy in premenopausal women with node-positive HR+HER2- BC and with a recurrence score(RS) lower than 25, also support the therapeutic endocrine effect of chemotherapy.[39] The observed lack of a progressive increase of benefit from chemotherapy with increasing values of the RS is more consistent with an indirect endocrine effect of adjuvant chemotherapy than with a direct cytotoxic effect, that is expected to be larger for tumors with higher RS values, characterized by higher proliferative rate and poorer endocrine-responsiveness.[39] Unfortunately, since most patients enrolled in the RxPONDER trial received only tamoxifen as adjuvant ET, uncertainty remains regarding the magnitude of benefit from adjuvant chemotherapy in premenopausal women when treated with OFS+AI.[39]

A second option to optimize OFS could be the use of GnRH-antagonist drugs. In men with prostate cancer, GnRH-antagonists achieve significantly faster and more effective castration as compared with agonists.[40,41] In the recent randomized phase II TREND trial, 51 premenopausal women with HR+HER2- early BC were randomized

to 6 months of neoadjuvant letrozole with the GnRH-agonist triptorelin or the GnRH-antagonist degarelix.[42]Achievement of optimal OFS was three times faster for patients assigned to degarelix, and OFS was maintained throughout the treatment period by all patients in the degarelix arm, whereas 15% of patients assigned to triptorelin had suboptimal OFS.[42]

The last suitable option to overcome suboptimal OFS may be offered by selective ER degrader (SERD) drugs. Indeed, while AIs are ineffective in case of suboptimal OFS, SERDs, similar to tamoxifen, retain some activity in case of suboptimal OFS.[43] Suboptimal OFS might be one of the possible reasons for the apparently inconsistent OS results between the SOFT trial, where OFS+AI led to slightly worse OS than OFS+tamoxifen, and the TEXT trial, where the opposite was observed. Patients in SOFT received their chemotherapy prior to enrollment and only those who did not have chemotherapy-induced ovarian failure were enrolled, while women in the TEXT trial were enrolled before chemotherapy was started, therefore, were more likely to have successful and maintained OFS than those of the SOFT trial.[34] Although evidence so far available indicates that Fulvestrant should be combined with OFS, since it exerts the highest antitumor activity in a low estrogen environment, it still has activity in case of poor estrogens suppression.[44] Furthermore, in two ongoing randomized neoadjuvant trials, the PREcoopERA and PremiERe trials, premenopausal women with stage I to III HR+/HER2- BCs, are randomized to a new oral SERD given as monotherapy or combined with OFS. The dynamic of Ki67 levels, assessed at baseline versus at surgery after 1 month of treatment, will be compared between treatment arms to test the hypothesis that new oral SERDs could be administered without OFS retaining similar antitumor biological effects. If such hypothesis will be confirmed, the new oral SERDs could substantially improve the outcome of patients at higher risk of suboptimal OFS, such as those at younger age or not receiving adjuvant chemotherapy.[28]

It must be considered, however, that the greater the depth and duration of estrogens deprivation achieved with such new therapeutic strategies, the higher is the risk of relevant adverse events (AEs). Premature surgical menopause has been associated with increased mortality in population studies.[45]Although currently there is no indication of excess of non-cancer deaths in patients assigned to OFS in SOFT trial as compared with tamoxifen, there was substantially higher risk of AEs of grade 3 or worse, including osteoporosis and fracture especially in the exemestane arm.[34] Thus, a careful selection of patients to escalate therapeutic strategies of estrogens suppression should be done, based both on predicted high risk of recurrence and on a multidimensional patient's health assessment, in order to achieve a positive therapeutic risk to benefit ratio.

Dissection of the biological effects of ER manipulation.

The role played by ERα in the pathogenesis of BC is multifaceted, and consequently the biological effects of its manipulation are complex and not fully understood.[46] Surprisingly, two relevant biological aspects of the therapeutic manipulation of ERα have been overlooked for decades.

The first is the reliable observation that in patients with metastatic BC, disease control can be obtained with both ERα blockade and activation. Before the introduction of tamoxifen, the synthetic estrogen diethylstilbestrol (DES) was the ET of choice for advanced BC. In the early 1980's a seminal RCT showed that tamoxifen was less toxic, although not more effective than DES, and DES use was therefore abandoned.[47] More recently, Ellis et al reported a clinical benefit rate of 28% and 29% in patients with metastatic BC therapeutically exposed to high and low estradiol levels, respectively.[48]

The paradoxical observation of obtaining the same antitumor effects with two opposite therapeutic interventions clearly highlights a gap in our understanding of the ERα role in BC pathogenesis.

To date, there is no clear interpretation on the biological mechanisms underpinning the antitumoral activity exerted by exogenous estrogens when administered in patients previously unexposed to hormonal manipulation. By contrast, some hypotheses exist on their effects when administered after extended estrogen deprivation. Jordan et al. provided elegant preclinical evidence showing that HR+BC cell lines with acquired-resistance to ERα-blockade after long-term estrogen deprivation, undergo apoptosis when re-exposed to estradiol.[49] Indeed, the molecular adaptation of cancer cells to low levels of estrogens might enhance the pro-apoptotic, endoplasmic-reticulum-stress and inflammatory responses when they are re-exposed to estrogens.[49] This observation was the biological rationale for the SOLE trial.[50] In this RCT, postmenopausal women without recurrence after 4-6 years of prior ET were randomized to receive either 5 additional years of continuous ET with letrozole, or intermittent letrozole with 3-months treatment interruption planned every 9 months to achieve physiological circulating E2-levels during therapeutic breaks. Although the results of the trial did not show higher efficacy of the intermittent approach, it is still possible that circulating E2-levels recovered after only 3 months of AI interruption were too low to obtain a meaningful antitumor effect.[50]

The POSITIVE trial is a single-arm phase II study designed to prospectively assess the safety of ET interruption to attempt pregnancy in premenopausal women with early HR+BC after 18 to 30 months of prior adjuvant ET.[51] Results of a secondary analysis of this trial showed a better outcome for women who became pregnant, and thus exposed to high circulating E2-levels, as compared with women who did not become pregnant. Although several biases (e.g, healthy-mother effect) or other possible explanations could play a role, this provocative observation further highlights the need to better explore this issue.[51]

The other poorly investigated aspect of ERα-manipulation in the context of adjuvant ET is its effects on non-cancer cells, particularly on the immune-system. ER directly regulates the expression of a number of immune-related genes, as well as the function of cells with a key role in anticancer immune-response (**Figure 1**).[52]

We and others showed that immune checkpoint inhibitors (ICIs) have significantly different efficacy in male and in female patients with several solid tumors. This sex-based difference is likely due to the profound modulation of the anticancer immune-response exerted by both androgens and estrogens.[52-54]

The extent to which ERα-blockade maintained for years in women who undergo adjuvant ET affects anticancer immune-response should be investigated for several possible therapeutic implications. Indeed, different types of ET may have substantially different effects on the immune-system: tamoxifen could exert context-dependent agonist and antagonist effects, whereas AIs and SERDs have only inhibitory effects on the ERα expressed by immune-cells. Furthermore, ET may also modulate ERβ that is widely involved in regulation of immune responses.[51] Evidence available showed that

both AIs and tamoxifen exert ERβ inhibition at clinically relevant concentrations, while the effects of SERDs on ERβ are less characterized.[55-57]

It might be promising to introduce ICIs in the (neo)adjuvant treatment of HR+/HER2- BC. Indeed, in preclinical BC models many complex immune-related effects of ERα-blockade have been reported, including upregulation of PD-L1 in multiple HR+BC cell lines contributing to T-cells evasion.[55]

Notably, positive results have been recently announced for the primary endpoint of pCR for the KEYNOTE-756 RCT, testing the combination of the anti-PD1 pembrolizumab with chemotherapy as neoadjuvant treatment, followed by adjuvant treatment with pembrolizumab plus ET in patients with high-risk, early-stage HR+/HER2- BC. However, since it has been shown that pCR is a poor surrogate

endpoint for DFS and OS at the trial-level for early-stage BC, and given the potential toxicity and financial burden of immunotherapy, mature results for the co-primary endpoint EFS should be awaited to drawn definitive conclusions on the potential efficacy of (neo)adjuvant ICIs for early-stage HR+/HER2- BC, and to assess the absolute amount of benefit provided on long-term patients' outcome.[58,59]

To date, conflicting results have been reported on the efficacy of ICIs in patients with advanced HR+/HER2- BC. In the PACE RCT, the addition of the anti-PD-L1 avelumab to fulvestrant and a CDK4/6-inhibitor, continued beyond progression, provides an intriguingly numerically longer PFS as compared with standard fulvestrant monotherapy in patients with advanced HR+/HER2- BC resistant to previous ET and CDK4/6 inhibitor.[60] On the other hand, in the SAFIR02 RCT, patients with advanced HR+/HER2- tumors had poorer OS when treated with durvalumab as maintenance therapy after an induction chemotherapy as compared with maintenance chemotherapy, in contrast to the patients with triple negative BC who obtained longer survival when treated with durvalumab.[61]

The SAFIR02 trial tested ICI as monotherapy, while a number of phase II trials are currently testing ICIs in combination with ET, mainly in patients with advanced HR+/HER2- BC but also in the neoadjuvant setting (**Table 2**).

Notably, in two different trials testing neoadjuvant AIs, poor Ki67 response to ET was correlated with high levels of TILs and multiple markers of high immune activity.[62,63] While there is no evidence that these associations are causative, they indicate that particular caution should be taken in applying stimulation of immunological response through ICIs in (neo)adjuvant setting for patients with ER+HER2- BC.

Refining patients' selection for treatment escalation.

Recently two large RCT, namely the MonarchE and NATALEE trials, consistently showed that the combination of CDK4/6 blockade with AIs significantly improved the prognosis of a patient population with HR+ BC selected for clinical pathological-features associated with intermediate and high-risk of relapse.[3,64]

Results of MonarchE and NATALEE trials are among the most important advances in the field of adjuvant ET of the past decade.[3,64] Indeed, the amount of iDFS benefit reported for the overall population included in the MonarchE trial is large enough to be considered clinically meaningful, and consequently, abemaciclib has been approved by global regulators and endorsed by international guidelines with a level 1 rating from the National Comprehensive Cancer Network and a maximum score for curative therapies from the European Society for Medical Oncology on the Magnitude of Clinical Benefit Scale. Consistent results have been recently reported at the second interim efficacy analysis of the NATALEE trial, testing ribociclib for three years in a patient population including not only node-positive tumors, as was done in the MonarchE trial, but also node-negative diseases with additional risk factors, including high histological grade, Ki67 labelling index or genomic risk.

Although mature results for OS are not yet available for either trial, two features of the iDFS KM-curves support the hypothesis that some of the iDFS benefit so far observed will ultimately translate in OS gain: i) the carryover protective effect of CDK4/6 inhibition beyond treatment interruption, resulting in progressive increasing of the iDFS benefit during the follow-up (the absolute improvement of the iDFS rate at 2, 3 and 4 years of follow-up was respectively 2.8%, 4.8%, and 6.4% in the MonarchE trial); ii) most of the iDFS benefit reported was due to the reduction of the risk of distant recurrence.

On the other hand, in the MonarchE trial the number of patients who needed to be treated (NNT) to prevent one additional recurrence was 15, and probably this number is even higher in the subgroup of patients with node-negative disease included in the NATALEE trial, considering their lower risk of recurrence. Obviously, the largest the NNT the higher the costs in terms of toxicity and financial burden not supported by

clinical benefit in a substantial group of patients. In both the MonarchE and NATALEE trials, subgroup analyses failed to identify any patient subgroup defined by baseline clinical-pathological features who benefit the most from CDK4/6 blockade.

All this highlights the urgent need to implement new approaches to improve the selection of patients as candidates for treatment escalation or de-escalation in the context of adjuvant ET.

The dynamic and in-vivo characterization of the biological effects of treatments on the tumor is one of the most promising approaches. The large prospective POETIC trial supported the feasibility and usefulness of such an approach.[65] Results of this trial showed that the combined evaluation of Ki67 levels assessed at baseline and after 2 weeks ($Ki67_{2W}$) of neoadjuvant ET with AIs, predicts individual patient outcome significantly better than baseline Ki67 alone.[65] Patients with high $Ki67_{2W}$ had a poor prognosis, and in the ongoing POETIC-A trial (NCT04584853) are randomized to receive a CDK4/6 inhibitor versus placebo in combination with adjuvant AIs.[65] The results of the POETIC-A trial will finally inform on the possibility of further refining the selection criteria of patients who might benefit from adjuvant ET escalation through the in-vivo characterization of tumor response to treatment, rather than only considering the baseline clinical-pathological characteristics of disease as done in the MONARCH-E and NATALEE trial.[3,64]

Similarly, the WSG-ADAPTcycle trial (NCT04055493), a phase III RCT, investigates whether patients with HR+/HER2- BC, identified as intermediate-risk based on Ki67 response after a short 3-week neoadjuvant ET, derive benefit from 2 years of adjuvant CDK4/6 blockade combined with ET without chemotherapy, as compared to patients treated with chemotherapy followed by standard ET.

The preoperative prognostic index (PEPI score) is a validated score, based on the response of the disease to neoadjuvant ET, that combines the Ki-67 level with ER

status, pathological tumor size and nodal status, to identify a subgroup of patients with endocrine-sensitive disease (i.e., PEPI score 0) and such a low rate of relapse with only ET that could be safely spared from adjuvant chemotherapy.[66]

The in-vivo assessment of disease response to neoadjuvant ET, has been recently implemented in the ALTERNATE trial, a large neoadjuvant RCT testing the hypothesis that the percentage of post-menopausal patients with PEPI score 0 could be improved with 6 months of neoadjuvant ET with fulvestrant or fulvestrant plus anastrozole as compared with anastrozole alone. Although no significant differences were reported between the three treatment arms, results of trial showed that such an approach can identify a subgroup of 25-30% of patients that can safely spare adjuvant chemotherapy.[67]

Another promising approach to identify patients who may benefit from adjuvant treatment escalation or de-escalation is to monitor for molecular/minimal residual disease (MDR) through circulating tumor DNA (ctDNA) detection during adjuvant ET.[68,69] Indeed, both retrospective and prospective evidence showed that detection of ctDNA in patients with early-stage BC, either immediately after surgery or mainly in subsequent serial sampling, is predictive of a higher risk of relapse, with a median lead time of 8-20 months prior to clinical recurrence.[70-73]

Two major issues affect MDR monitoring in patients with surgically resected early-stage tumors. The first is that the overall ctDNA amount is proportional to the tumor burden. As opposed to the metastatic setting, where ctDNA may represent the large majority of circulating free DNA (cfDNA), in the context of MDR the ratio of ctDNA/cfDNA is frequently lower than 0.1% - 0.01%, thus requiring assays with very high sensitivity.[68,69] The second issue, is the not negligible rate of false positive findings, due in part to technical errors and in part to biological issues, such as the clonal hematopoiesis of indeterminate potential (CHIP), a key confounder in ctDNA analysis.[68,69]

In the last years, two main approaches led to a reliable assessment of MDR in patients with early-stage BC. The first was the refinement of tumor-agnostic assays, initially developed for the detection of ctDNA in metastatic settings, and now substantially improved for use in the low-ctDNA context. The majority of such tumor-agnostic assays rely on ultra-deep targeted sequencing of a panel of known cancer-associated alterations via next-generation sequencing (NGS). The very deep coverage of a large panel of genomic alterations (including single-nucleotide variants, insertion-deletion and copy number alterations), and for some assays also epigenomic alterations (such as aberrant DNA methylation patterns), allow yielding high sensitivity while retaining meaningful specificity of results.[68,69]

The second approach is a "tumor-informed" ctDNA assessment, that monitors for a known set of tumor-specific alterations. Tumor-informed approaches involve analyzing patient's tumor and healthy tissue/blood samples through ultra-deep whole-exome or whole-genome sequencing to identify somatic alterations specific to that exact patient's tumor and distinct from other cfDNA, including germline variations.

As compared with tumor-agnostic, tumor-informed approaches have several advantages, such as higher sensitivity and specificity, but also some disadvantages, such as longer turnaround time, and limited ability to assess the genomic evolution of tumors, since most assays only detect a limited number of tumor-specific alterations, including non-pathogenetic variants. [68,69]

Several ongoing RCTs are testing whether the addition of CDK4-6 inhibitors to adjuvant ET at the time of detectable MDR but without evidence of metastatic disease on radiological assessment, can improve patients' prognosis. The majority of these trials share a similar two-phase design: the first, non-randomized and surveillance phase, aims to validate the use of ctDNA to predict the risk of relapse in patients with early-stage HR+/HER2- BC who are receiving adjuvant ET; in the second, randomized and interventional phase, patients with positive ctDNA detection and no macroscopic disease on the staging scan, will be randomized to continue ongoing adjuvant ET as per standard clinical practice, versus an experimental

treatment including CDK4-6 inhibitors. [68,69] In the LEADER (NCT03285412) and DARE (NCT04567420) RCTs, ribociclib and palbociclib will be respectively added at the ongoing adjuvant ET with tamoxifen or AI, while in the TRAK-ER trial (NCT04985266) patients in the experimental arm will be switched to treatment with fulvestrant plus palbociclib. In all these three trials, the ctDNA will be assessed through a "tumor-informed" approach.

MDR detected late during patients' follow-up may also inform the optimal duration of adjuvant ET. Recently, Lipsyc-Sharf et al. reported an analysis of 83 patients with no evidence of disease recurrence 5 years after diagnosis and prospectively followed-up through tumor-informed ctDNA. Results showed that 10% of patients had positive ctDNA detection at any time point during the study, and that ctDNA detection anticipated distant recurrence in all cases with a lead time of 12.4 months. Even if larger studies are needed to confirm these findings, detection of ctDNA at the end of the standard 5 years of ET might indicate the need to extend adjuvant ET, and in some cases might also inform therapeutic switch to specific therapies able to overcome the mechanisms of resistance identified, such as the emergence of *ESR1*-mutations targetable by new oral SERDs.[27,28]

Despite the evidence available supporting MDR monitoring as a promising tool to inform adjuvant treatment escalation/de-escalation in patients with early-stage BC, several important questions remain to be addressed.

For example, while it has been shown that serial testing substantially improves the sensitivity of MDR detection, no evidence is available on the best sampling time schedule.

Furthermore, in some contexts the sensitivity of MDR detection remains limited, such as isolated local recurrence (ILR) or isolated CNS metastases, likely due to low levels of ctDNA, given the low tumor burden in the first case and the anatomic limitations of the blood-brain barrier in the latter. [68,69]

Given that patients with BC do not undergo systemic radiographic surveillance after curative surgery, to date evidence available only showed that MDR detection allows for significantly anticipate diagnosis of disease recurrence as compared with symptomatic clinical recurrence, while a robust demonstration as compared with asymptomatic recurrence detected through radiographic surveillance is still missing. Notably, in the c-TRAK-TN trial, a phase II trial aimed to evaluate the efficacy of pembrolizumab in the setting of MRD after treatment for early-stage TNBC, over half of patients with MRD in fact had metastatic disease on staging scans at the time of ctDNA detection.[74]

Most importantly, demonstrating that treatment intervention started at the time of MDR detection can modify the natural history of the disease, eradicating micrometastases and ultimately improving patients' prognosis, raises methodological challenges. Indeed, many ongoing RCTs testing therapeutic interventions started at MDR detection, have DFS as the primary endpoint, while its surrogacy value for OS in this context is completely unknown. Indeed, drugs administered at MDR detection may simply delay the time to clinical recurrence without eradicating micrometastases, and thus may not improve the overall survival of patients as compared with starting the same treatments at the time of clinical recurrence. Thus, RCTs with OS as the primary endpoint should be performed in this context, at the cost of substantially larger sample sizes and longer follow-up.

Conclusions.

After decades of research, improving the efficacy of adjuvant ET becomes increasingly difficult. We discussed some key principles that should inform future research to achieve this objective, and some representative examples on how to pursue them in poorly investigated areas. In our opinions, the most promising opportunities to enable better tailoring of ET for patients with HR-positive disease include i) implementation of technological breakthroughs, such as ctDNA assays to identify patients with residual molecular disease during and after adjuvant ET, ii) the

definitive validation of clinico-pathological surrogate endpoints of DFS for HR+BC, such as the PEPI or CPS+EG scores to accelerate drug development through neoadjuvant trials, and iii) the development of more representative preclinical models, such as immunocompetent humanized mice to fully capture the ET effects on both cancer cells and the immune-system.[75,76]

Figure 1. Pro-tumor immune-related effects exerted by estradiol (E2) and Estrogen receptor (ER) pathway activation. A) E2/ER pathway polarizes macrophages towards a M2 phenotype associated with tumor progression; B) E2/ER pathway promotes the tumor infiltration and activation of neutrophils with an increased expression of pro-tumor chemokines (e.g. CXCL-1, and CXCL-2) and tissue-remodeling enzymes (MMP-3, MMP-9 and COX-2); C) E2/ER pathway induces expression of the granzyme B inhibitor protease inhibitor 9 (PI-9) by target cells, including BC cells, that hampers NK cells cytotoxic activity; D) E2/ER pathway impairs antigen presentation by mature DCs through decreased secretion of cytokines (e.g. IFNγ, IL-12 and TNFα); E) E2/ER pathway directly inhibits the proliferation of CD4 + T cells through reduced expression of both IL-2 and IL-2R. (Adapted from an image created with BioRender.com.)

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Table 1. Main features of trials discussed

Trial Name	Phase	Patients population	Setting	N of pts	Menopausal status	Control arm	Experimental arm	Primary out.	Results
MonarchE	III	HR+, HER2-, high-risk EBC	adjuvant	5637	pre and post	ET	ET + 2 yrs abe.	iDFS	HR: 0,6 (95%CI, 0.5-0.7)
NATALEE	III	HR+, HER2-, stage II and III	adjuvant	5101	pre and post	ET	ET + 3 yrs rib.	iDFS	HR: 0.75 (95%CI, 0.62- 0.9)
aTTom	III	HR+ or unknow EBC, treated with 5 years of tam.	adjuvant extended	6953	pre and post	placebo	tam. x 5 yrs	BCR	RR yrs 5-6: 0.99 (95%CI, 0.86-1.15); RR yrs 7-9: 0.84 (0.73-0.95); RR >10yrs: 0.75 (0.66-0.86);
ATLAS	III	HR+ EBC, treated with 5 years of tam.	adjuvant extended	6846	pre and post	placebo	tam. x 5 yrs	BCR	RR yrs 5-9: 0.90 (95%CI, 0.79-1); RR >10 yrs: 0.75 (0.62-0.9);
MA17	III	HR+ EBC, treated with 5 years of tam.	adjuvant extended	5187	post	placebo	let. x 5 yrs	DFS	HR: 0.58 (95%CI, 0.45-0.76)
NSABP-42	III	HR+ EBC, treated with 5 yrs of let. or tam.->let.	adjuvant extended	3966	post	placebo	let. x 5 yrs	DFS	HR: 0.84 (95%CI, 0.74-0.96)
SALSA	III	HR+ EBC, treated with 5 yrs of AI or tam->AI	adjuvant extended	3484	post	let. x 2 yrs	let.x 5 yrs	DFS	HR: 0.99 (95%CI, 0.85-1.15)
IDEAL	III	HR+ EBC, treated with 5 years of AI or tam->AI	adjuvant extended	1824	post	let. x 2.5 yrs	let. x 5 yrs	DFS	HR: 0.92 (95%CI, 0.74 to 1.16)
DATA	III	HR+ EBC, treated with 2-3 years of tam.	adjuvant extended	1912	post	ana. x 3 yrs	ana. x 9 yrs	DFS	HR: 0.79 (95%CI, 0.62-1.02)
OlympiA	III	HER2- and BRCA1/2 mutated, high-risk EBC	adjuvant	1863	pre and post	placebo	olaparib x 1 yrs	iDFS	HR: 0.58 (95%CI, 0.41-0.82)
TEXT and SOFT	III	HR+ EBC	adjuvant	4690	pre and post	OFS+tam. x 5 yrs	OFS+exe. x 5 yrs	DFS	HR: 0.79 (95%CI, 0.70-0.90)
RxPONDER	III	HR+/HER2- EBC, with 1-3 positive nodes and RS<25	adjuvant	5083	pre and post	ET	cht + ET	iDFS	HR in postmenopausal pts: 1.02 (95%CI, 0.82-1.26); HR in premenopausal: 0.6 (95%C,I 0.43-0.83)
TREND	single arm II	HR+/HER2- EBC	neoadjuvant	51	pre	triptorelin + let. x 6 mo.	degarelix + let. x 6 mo.	t.t.o. OFS	HR: 3.0 (95%CI, 1.6-5.6)
Tam vs DES	III	advanced BC in first or further lines	metastatic	143	post	DES	tam.	ORR	41% vs 33% p=0.5
HD vs LD E2	randomized II	HR+ advanced BC, treated with AI	metastatic	66	post	30 mg/daily E2	6 mg/daily E2	CBR	28% (95%CI, 18%-41%) vs 29% (95%CI, 19%-42%)
SOLE	III	HR+ EBC, treated with 5 yrs of let. or tam.->let.	adjuvant extended	4884	post	continuous let. x 5 yrs	intermittent let. x 5 (9 mo. on/3 mo. off)	DFS	HR: 1.03 (95%CI, 0.91-1.17)
POSITIVE	single arm II	HR+ EBC, after 18-30 months of adjuvant ET	adjuvant	516	pre (<42 years)	NA	Up to 2 yrs of ET interruption	BCFI	3-yr BCFI: 8.9% (95%CI, 6.7-11.9)

PACE	Randomized II	HR+/HER2- advanced BC, resistant to ET+CDK 4/6 inh.	metastatic	220	pre and post	Ful.	Ful. + CDK4/6 or Ful.+CDK4/6+Ave	PFS	Ful. +CDK4/6 vs Ful. HR: 1.1 (95%CI, 0.7-1.6) Ful. +CDK4/6+Ave vs Ful. HR: 0.75 (95%CI, 0.4-1.2)

Abbreviations.
abe.: abemaciclib; AI: aromatase inhibitor; ana: anastrozole; Ave.: Avelumab; BCFI: breast cancer free interval; BCR: Breast cancer recurrence; CBR: clinical benefit rate (i.e., complete or partial response or stable disease > 24 weeks); CDK4/6 inh.: Cycline dependent kinase 4/6 inhibitor; E2: estradiol; exe: exemestane; Ful.: Fulvestrant; HD: high dose; HR: Hazard Ratio; HR+: Hormone receptor positive; iDFS: invasive disease free survival; LD: let.: letrozole; low dose; mo: month; OFS: ovarian function suppression; rib: ribociclib; ORR: overall response rate; RR: relative risk; tam: tamoxifene; t.t.o OFS: time to optimal OFS (i.e., time from the first injection to first assessment of centrally assessed estradiol level ≤ 2.72 pg/mL [≤ 10 pmol/L] during neoadjuvant therapy); yrs: years;

Table 2. Ongoing trials testing endocrine therapy combined with immunotherapy

Clinicaltrials.gov identifier	Treatment arms	Phase	Patients population	Sample size	Setting	Primary Outcome
NCT03879174	Pembrolizumab + Tamoxifen	single arm, phase II	HR+/HER2- advanced BC, with ESR1 mutation and resistant to AI	25	advanced	PFS, ORR
NCT03393845	Pembrolizumab + Fulvestrant	single arm, phase II	HR+/HER2- advanced BC	47	advanced	CBR
NCT02990845	Pembrolizumab + Exe.+ Leuprolide	single arm, phase II	Premenopausal women with HR+/HER2- advanced BC, resistant to first line ET	25	advanced	PFS
NCT03874325	Durvalumab + AI	single arm, phase II	HR+/HER2- early BC	17	neoadjuvant	mPEPI
NCT03280563	Fulvestrant compared with 1) Atezolizumab + Fulvestrant 2) Atezolizumab + Ipatasertib 3) Atezolizumab + Ipatasertib + Fulvestrant 4) Atezolizumab + entinostat	randomized, umbrella, Phase Ib/II	HR+/HER2- advanced BC, resistant to ET + CDK4/6 inh.	138	advanced	ORR
NCT02778685	Pembrolizumab + Palbociclib + Let. or Fulvestrant	single arm, phase II	postmenopausal women and men with HR+/HER2- advanced BC	47	advanced	CR, PR
NCT04088032	Abemaciclib + Durvalumab + AI	single arm, phase II	premenopausal women with HR+/HER2- early BC	NA	neoadjuvant	rate of AEs
NCT04075604	Palbociclib + ana. versus Nivolumab + Palbociclib + Ana.	randomized, non-comparative phase II	postmenopausal women and men with HR+/HER2- early BC	23	neoadjuvant	DLT, RCB

Abbreviations:
Ana: anastrozole; Exe: Exemestane Let: letrozole; AI: aromatase inhibitor; mPEPI: modified Preoperative Endocrine Prognostic Index; RCB: Residual Cancer Burden; AEs: adverse events; DLT: dose limiting toxicities; PFS: progression free survival; ORR: overall response rate

Figure 1