REVIEW

# Tumour growth and immune evasion as targets for a new strategy in advanced cancer

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## Abstract

It has become clearer that advanced cancer, especially advanced breast cancer, is an entirely displayed pathological system that is much more complex than previously considered. However, the direct relationship between tumour growth and immune evasion can represent a general rule governing the pathological cancer system from the initial cancer cells to when the system is entirely displayed. Accordingly, a refined pathobiological model and a novel therapeutic strategy are proposed. The novel therapeutic strategy is based on therapeutically induced conditions (undetectable tumour burden and/or a prolonged tumour 'resting state'), which enable an efficacious immune response in advanced breast and other types of solid cancers.

**Key Words**
- breast cancer
- immune evasion
- tumour growth
- cancer therapy

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## Introduction

As testified by ancient writers, human beings have always been suffering from cancer. It is likely that environmental pollution and prolonged ageing concomitant with some radical lifestyle changes (Ferlay *et al.* 2012, Howell *et al.* 2014) are among the main reasons for the increasing prevalence of cancer in the modern era. In its advanced stages, cancer is often an incurable disease and represents a serious threat to human life. In 2012, the International Agency for Research on Cancer reported 14.1 million new cancer diagnoses, 8.2 million cancer deaths and 32.6 million cancer diagnoses of <5 years worldwide. The cancer death rate ranges 69–173 per 100,000 men and 65–119 per 100,000 women (Ferlay *et al.* 2012). Thus, cancer has now acquired a major social relevance. Among women, breast cancer is the most common cancer in most regions of the world, with an estimated incidence of 246,000 new cases (29% of all cancer cases) and 40,450 deaths (14% of all cases) in 2016 in the United States (Siegel *et al.* 2016). This paper reviews some recent experimental and clinical data to propose an innovative therapeutic strategy for advanced breast and other cancers based on the relationship between tumour growth and immune evasion.

## The biological cancer hallmarks and the current model

In 2011, Hanahan and Weinberg published an update (Hanahan & Weinberg 2011) of their milestone article on the principal hallmarks of cancer (Hanahan & Weinberg 2000). The authors reported that the principal biological capabilities acquired by cancer cells are sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative

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immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy and metabolism and evading immune destruction. In this updated model, genomic instability and inflammation are the basis of all the hallmarks. The network sustaining cancer growth and progression is represented as an overall integrated circuitry comprising a few interconnected subcircuits. In turn, each subcircuit comprises multiple interconnected pathological molecular pathways fostering different hallmark capabilities. Here, we focus on advances in the role of tumour growth and immune evasion in tumour progression and diffusion.

## Tumour growth

### Sustaining proliferative signalling

The growth-promoting signals in cancer cells are mainly induced by growth factors that bind cell surface receptors with intracellular tyrosine kinase domains. In addition, growth factors acquire the capability to carry mitogenic signals in some different ways. In particular, autocrine or paracrine proliferative stimulations and downstream molecular pathways, either constitutively activated or activated following somatic mutations, are common (Davies & Samuels 2010, Nicolini *et al.* 2015). Many other mechanisms of tumour growth promotion (Aziz *et al.* 2015, Gao *et al.* 2015*a*, Rohatgi *et al.* 2015, SPN *et al.* 2015, Wang *et al.* 2015*a*) have been reported. Some of these studies (Song *et al.* 2015, Wang *et al.* 2015*c*, Koval *et al.* 2016, van Geldermalsen *et al.* 2016) included triple-negative cancer subtype.

### Evading growth suppressors

In recent years, a few tumour suppressor genes and the inhibition of anti-proliferative mechanisms have been identified (Kochupurakkal *et al.* 2015, Ma *et al.* 2015). The constitutive activation of the interferon gamma (IFNγ)/signal transducers and activators of transcription (STAT) 1/interferon-regulatory factor (IRF)-1 axis (T-helper (Th) 1 phenotype) correlates with good prognosis and predicts better response to anti-cancer therapy (Ascierto *et al.* 2011). IRF-1 regulates the transcription of a set of target genes that play principal roles in tumour immune surveillance and immune system development. The mechanisms by which IRF-1 mediates tumour suppression are not clear; however, several IRF-1 target genes that inhibit growth by cell cycle arrest and promote apoptosis have been identified (Dou *et al.* 2014). Many reports have suggested a relevant role of IFNγ/STAT1/IRF-1 axis in the endocrine resistance of oestrogen receptor (ER)-positive breast cancer cells (Clarke *et al.* 2009, Ning *et al.* 2010, Schwartz *et al.* 2011, Schwartz-Roberts *et al.* 2015). Yang *et al.* (2017) recently showed that the suppression of the immune functions of T cells in the tumour microenvironment (TME) is another mechanism by which oestrogen drives cancer progression. Accordingly, in two experimental studies, IRF-1- and IFNγ-mediated apoptosis was induced by the anti-oestrogens tamoxifen (Bowie *et al.* 2004) and fulvestrant (Bouker *et al.* 2014). These observations also suggest changes in gene expression from heterogeneous tumour samples. In breast cancer, additional mechanisms regarding the evasion of tumour growth suppressors (Hu & Xie 2015, Xu *et al.* 2015, You *et al.* 2016) have been described.

### Resisting cell death

In principle, cell death, mainly by apoptosis or necrosis, is thought to be a main natural hindrance to cancer development. Apoptosis plays a fundamental role in the homeostasis of healthy tissues. In the last decades, it has been fully elucidated how apoptosis is triggered in response to different physiological stimuli. The apoptotic machinery is governed by upstream regulators and downstream effectors, and the regulators include two major circuits: the extrinsic and intrinsic apoptotic programmes (Cory & Adams 2002, Kiraz 2016). Recently, several mechanisms that can affect apoptosis in breast cancer cells have been described (Sayeed *et al.* 2013, Armstrong *et al.* 2015, Farrugia *et al.* 2015, Gao *et al.* 2015*b*, Han *et al.* 2015, Li *et al.* 2015, Liu *et al.* 2015, Saqcena *et al.* 2015, Zhou *et al.* 2015, Cao *et al.* 2016, Shrestha *et al.* 2016).

### Enabling replicative immortality

Other investigational findings on senescence (El Hasana *et al.* 2015) and autophagy (Artero-Castro *et al.* 2015) have been reported. Autophagy is a 'self-eating' process initiated by cancer cells in response to various stresses. Both autophagy upregulation and downregulation have been found in cancer, suggesting its dual oncogenic and tumour-suppressing roles during malignant transformation (Marinković *et al.* 2018). However, in the last decades, accumulating evidence by experimental studies has indicated the relevance of autophagy in cancer progression and diffusion. These studies have elucidated further mechanisms of autophagy in human ER-α+ (Galindo-Moreno *et al.* 2017, Hou *et al.* 2017, Leignadier *et al.* 2017, Wang *et al.* 2017) or ER-α+ and ER-α–

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(Han *et al.* 2017*a*, Lin *et al.* 2017, Zhou *et al.* 2017*a*,*c*) breast cancer cells. In addition, they found that cytotoxic (chemo or endocrine) treatment (Kondo *et al.* 2005, Chen *et al.* 2011) and severe hypoxia (Rouschop 2010) are two major stresses that could be evaded by autophagy. In fact, in both cases, autophagy allows the cancer cell to survive and become refractory to chemo-endocrine therapy and chemoradiotherapy. Other translational research studies (Ueno *et al.* 2016, Han *et al.* 2017*b*, Tavera-Mendoza *et al.* 2017, Zhou *et al.* 2017*b*) have been conducted to identify novel prognostic biomarkers or key targets for developing new therapeutic agents. Some clinical trials using the autophagy inhibitors chloroquine (CQ) or hydroxychloroquine (HCQ) are ongoing. Two of them (NCT02333890 and NCT01023477) are evaluating CQ efficacy in decreasing tumour growth prior to surgical intervention, and one (NCT01446016) is evaluating CQ efficacy when given in combination with taxane in metastatic setting in patients who had previously failed to respond to anthracycline chemotherapy. In two trials (NCT03032406 and NCT03400254), HCQ alone or with everolimus or gedatolisib has been administered for preventing recurrent breast cancer. In another trial (NCT00765765), HCQ has been administered in a metastatic setting in combination with ixabepilone vs ixabepilone alone, with the decrease in tumour growth and response rate being evaluated as the main end points. All these trials have recruited breast cancer patients independent of hormone receptor status. A further ongoing trial enrolling ER+ patients (NCT02414776) is evaluating the response rate following the addition of HCQ in patients showing progress with hormonal therapy.

## Immune evasion

### Mechanisms of immune suppression or immune escape

Indeed, it is now a consolidated concept that during tumour development, a chronic inflammatory microenvironment reduces the anti-tumoural immune response and favours the escape of tumour from immune elimination (Clevers 2004, Bui & Schreiber 2007). Inflammatory immune cells include tumour-associated macrophages (TAMs), cytotoxic T (CD8) lymphocytes (CTLs), Th (CD4) lymphocytes, natural killer (NK) cells, regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs). Among them, Treg cells, MDSCs and macrophages are mainly involved in the immunosuppressive action (Vasaturo *et al.* 2015) via the secretion of key molecules, such as transforming growth factor beta (TGF-β), prostaglandin E2, indoleamine 2,3-dioxygenase and interleukin (IL)-10 (Capietto *et al.* 2011). The abundance of Tregs, MDSCs and TAMs in the stroma also helps cancer cells to escape immune surveillance and is associated with worse prognosis (Mantovani *et al.* 2006, Greten *et al.* 2011, Bergenfelz *et al.* 2015, Li *et al.* 2018), whereas CTLs are associated with a good prognosis (Tosolini *et al.* 2011). Signals derived from cancer cells and the stroma determine the TAM phenotype from M1, which stimulates immunoprotective inflammatory responses, and M2, which has an immunosuppressive action. M2 phenotype is found in most tumours where the TAMs induce angiogenesis, tumour growth and metastasis by secreting soluble mediators, cytokines and chemokines and by directly interacting with cancer stem cells (CSCs) (Hao *et al.* 2012). Soluble mediators, mainly growth factors, cytokines and chemokines, in addition to host immune cells are also produced by cancer-associated fibroblasts (CAFs) or by tumour cells themselves. Several growth factors, namely TGF-β, insulin-like growth factor 2 (IGF-2) and vascular endothelial growth factor (VEGF), cytokines, namely IL-1, IL-4, IL-6, IL-8, IL-10 and tumour-necrosis factor alpha, chemokines, namely chemokine (C-X-C motif) ligand 1 and C-C motif chemokine receptor 7, have been reported to be closely involved in tumour progression, invasion and immune escape (Eftekhari *et al.* 2017, Gál *et al.* 2017, Setrerrahmane & Xu 2017) and are potential targets for anti-tumour therapies. Moreover, cancer cells not only express these soluble mediators but also frequently overexpress the related receptors to escape from the immune responses (Setrerrahmane & Xu 2017). As is well known, tumour antigens must be presented in a human leucocyte antigen (HLA)-restricted way to be recognized by T-cell receptors. Impaired HLA-I or HLA-II expression prevent the activation of cytotoxic immune cells or affect the antigen-presenting capability of antigen-presenting cells. In addition, aberrant HLA-G expression by cancer cells inhibit the activity of all immune cells. These HLA-associated immune evasion mechanisms occur early and frequently in most cancer types (McGranahan *et al.* 2017, Rodríguez 2017). Nod-like receptor family caspase recruitment domain-containing 5 (NLRC5) has been found to be a crucial transcriptional co-activator of major histocompatibility complex (MHC) class I gene expression. NLRC5 expression strongly correlates with genes in the MHC class I antigen presentation pathway, including transporter associated with antigen processing (TAP) 1. In different types of cancer, epigenetic and genetic alterations are most prevalent in NLRC5 among all the MHC class I-related genes and are associated with impaired expression of the MHC I pathway components

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and immune evasion (Yoshihama *et al.* 2016). Accordingly, TAP1 downregulation has been shown to elicit immune escape in colorectal cancer (Ling *et al.* 2017). Further described mechanisms of immune evasion in breast and other cancer cells involve increased programmed death-ligand 1 (PD-L1) expression (Coelho *et al.* 2017, Martinez *et al.* 2017), stabilized PD-L1 mRNA (Glodde & Hölzel 2017) and altered PD-L1 function (Maj *et al.* 2017) by different molecular pathways. Nuclear factor (NF)-kb and increased PD-L1 expression are involved in immune evasion and the progression of triple-negative breast cancer (TNBC) (Maeda *et al.* 2018). The expression of the enzyme arginase 1 (ARG 1) as a key mediator of immune suppression (Steggerda *et al.* 2017) and the loss-of-function Janus-activated kinase (JAK) 1 mutations suggestive of immune suppression (Albacker *et al.* 2017) have also been reported in multiple cancer types. Moreover, other recent studies have reported on immune evasion (Hix *et al.* 2011, Khaled *et al.* 2013, Markosyan *et al.* 2013, Loumagne *et al.* 2014, Tao *et al.* 2014, Virtanen *et al.* 2014, Olesch *et al.* 2015, Zhang *et al.* 2015*b*,*c*, Zelenay *et al.* 2015, Gameiro *et al.* 2016, Heng *et al.* 2016, Lim *et al.* 2016, Loi *et al.* 2016).

## Probable reasons for the discrepancy between genetic and biological advances and clinical outcome

Despite the vast acquired biological knowledge, advanced breast cancer remains a disease with poor prognosis. Currently, endocrine therapy, chemotherapy and, more recently, the so-called 'targeted therapies' are common medical treatments for the advanced disease stages. Despite the availability of new biological drugs and a more rational use of therapies, the clinical outcome remains poor. Thus, the life expectancy of patients with advanced disease is dismal, and the median survival of a mixed population of metastatic breast cancer patients has not substantially improved in the last decades (Chia *et al.* 2007, Dawood *et al.* 2008, Cheng *et al.* 2009, Welt *et al.* 2016, Cardoso *et al.* 2017, Toss *et al.* 2017).

### The genetic and epigenetic heterogeneities

To date, cDNA and mRNA sequencing has allowed several 'genetic signatures' and molecular profiles to be defined, which usually differ in different tumours and different samples of the same tumour (spatial intratumour heterogeneity) (Yachida *et al.* 2010, Verigos & Magklara 2015). Each of these multigene sets is different in terms of the number and, at least in part, the genes involved. Moreover, although the most relevant genes for predicting patient outcome are those involved in cell proliferation, only Oncotype DX (Paik *et al.* 2004) and Mammaprint (van de Vijver *et al.* 2002) can be routinely recommended for predicting response to a specific type of therapy (Duffy *et al.* 2017). cDNA heterogeneity is increased further by epigenetic alterations and their ability to regulate gene expression (silencing oncogenes or activating repressor genes). The relevance of the contribution of epigenetic alterations to the genetic cancer heterogeneity became clear when hypo/hypermethylation of DNA and micro RNA (miRNA) function were investigated. Many patterns of methylation changes (hypo or hyper) have been described to be associated with different canonical pathways (Rodenhiser *et al.* 2008) or sometimes corresponding to different known genetic signatures. Many upregulated or downregulated miRNAs, numerous oncogenic and tumour-suppressive miRNAs involved in relevant molecular pathways, such as proliferation and survival, cell migration and metastasis, CSC phenotypes and epithelial-to-mesenchymal transition (EMT) processes, have been reported (Bertoli *et al.* 2015, Wang *et al.* 2015*b*). Currently, breast cancer classification into molecular subtypes considers the genetic characteristics of tumour. If the epigenetic alterations were also taken into account, many more subtypes could be generated. This inconsistency indicates the existence of further different molecular subtypes of breast cancer and even more complex classifications than are currently known.

### The genomic instability and the plasticity of phenotypes

Genomic instability is a prominent property of cancer cells that allows them to accumulate random mutations over time to acquire and better orchestrate their hallmark capabilities. The accumulation of mutations occurs due to the naturally developing genetic aberrations combined with those following the selective pressure of anti-cancer treatments (Zardavas *et al.* 2015). Tumour stroma is also involved in genomic instability. In a study of 51 breast cancer gene (BRCA)1/2-related cancers and 134 sporadic breast cancers, the accumulation of genomic instability in the tumour stroma corresponded to that in the neoplastic epithelium (Weber *et al.* 2006). In another study, human orthologs of genes identified in the stromal reaction to tumour progression in a mouse model were also expressed in several human cancers (Bacac *et al.* 2006). These and other findings indicated that genomic instability induces

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stromal alterations capable of promoting neoplastic transformation and stimulating tumour progression. In addition, genomic instability is favoured by the compromised surveillance system that normally detects and resolves defects in DNA or forces genetically damaged cells into senescence or apoptosis (Kastan 2008, Jackson & Bartek 2009). Recently, genetic differences have been shown between a primary breast tumour and its associated metastatic lesions, which developed over time (Zardavas *et al.* 2015). Moreover, sequencing data from cell populations as well as from single cells have shown three classes of mutations, namely (a) clonal mutations observed in the population sample and in most single tumour cells, (b) subclonal mutations found only in single cells and not in the population and (c) *de novo* mutations observed in one tumour cell only (Wang *et al.* 2014). These findings indicate that there is significant tumour heterogeneity, even at the single-cell level and suggest that different tumour subclones are the result of the accumulation of different point mutations over time (Wang *et al.* 2014, Wang & Navin 2015). Thus, genomic instability of stroma and cancer cells accounts mainly for temporal intratumour heterogeneity and describes tumour evolution. Biological plasticity is another important feature that significantly contributes to temporal tumour heterogeneity during cancer progression. Therefore, an initially more genetically homogeneous population of cells within a tumour becomes phenotypically heterogeneous due to the presence of cells in distinct states of differentiation following phenotypic variability, at least in part. The main example of this biological plasticity is the phenotypic variability implicit in CSCs where the activation of an EMT (Kalluri & Weinberg 2009) or endothelial-to-mesenchymal transition (Potenta *et al.* 2008, Mihira *et al.* 2012) programme converts epithelial or resident fibroblasts or endothelial cancer cells into mammary cancer cells (MCCs) or CAFs. On the other hand, programmes that convert endothelial cells to mesenchymal cells or mesenchymal cells to endothelial cells have been documented within stroma (Medici *et al.* 2010). Although all of these programmes and the contextual signals tend to promote an invasive tumour phenotype, in the absence of exposure to these signals, cancer cells may also revert to a non-invasive state through a process termed mesenchymal–epithelial transition (MET). This process is associated with cancer progression and metastasis. At the site of metastases, mesenchymal tumour cells must undergo MET as metastases recapitulate the pathology of the corresponding primary tumours. Thus, 'the cellular plasticity, the ability to undergo EMT and, subsequently, MET in the appropriate microenvironment, is a key feature of a successful metastatic cell' (Hugo *et al.* 2007). Moreover, cells do not complete these transformation programmes and frequently acquire a few traits of the new phenotype while continuing to express residual traits of the old phenotype. This contributes to increased temporal tumour heterogeneity.

## Incomplete knowledge of the mechanisms and the 'contextual signalling' that affect pathological molecular pathways sustaining the cancer hallmarks

The deficiencies in the knowledge and the complexity of the mechanisms sustaining tumour growth are well known to the investigators and are also clearly mentioned in the updated article by Hanahan & Weinberg (2011). In a recent review (Smithson *et al.* 2016), it has been suggested that 'signalling represents the language of the cell, where molecules (words) and cellular context (syntax) serve as units of informational content'; in addition, the authors stated that 'when we study signalling pathways in normal cells or in the setting of cancer, we often fail to consider how the cellular language conferred by these pathways is influenced by context, that is, the different extracellular signals present in the immediate milieu, the various adaptive responses that limit and promote intracellular signal transduction, the innate properties of distinct cell types responding to these cues, and the impact of epigenetic/genomic changes on the ultimate consequence of these informational signals'. They concluded that 'a deeper appreciation of contextual signalling may improve our understanding of the basic principles that govern development'. Recently, other authors have stated that 'a direct approach of inhibiting single oncogenic proteins misses the dynamic network context governing the network signal processing' (Fey *et al.* 2016). Overall, these deductions demonstrate that the comprehension of cancer is a work in progress.

## Chemo, hormone and targeted therapies: main limitations

Locally confined primary cancer is commonly called 'early' or 'advanced cancer' according to whether, at the time of diagnosis, it corresponds to the initial or successive stages of the ongoing internationally recognized clinicopathological classifications. Regional involvement (regional lymph nodes or regions around the primary cancer) makes any locally confined primary cancer an advanced cancer. When organs that are distant

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from the site of primary cancer are involved, cancer is called metastatic, and even metastatic disease constitutes advanced cancer. Although surgery and radiotherapy function loco-regionally, conventional chemo, hormone and targeted therapies are directed to cancer cells wherever they are present in the body tissues. Therefore, they are usually administered to patients with advanced cancer to prevent (adjuvant therapy) or treat (salvage therapy) metastatic disease. However, the development of acquired resistance and toxicity are two limiting aspects common to all therapies, although they generally differ according to the type of drug. In hormone-sensitive patients, hormone therapy is very rarely interrupted by heavy toxicity (Nicolini *et al.* 2016). It is likely that the absent or mild side effects and a more prolonged efficacy reflect the limited number of normal tissues involved in addition to cancer cells (Couse & Korach 1999) as well as the more terminal inhibition of multiple transduction signalling pathways activating the targeted intracellular biological processes respectively. Usually, because of the lack of significant efficacy when administered alone, the targeted therapies are given in combination with chemotherapy or hormone therapy. Nevertheless, patients receiving targeted therapies often exhibit moderate or heavy toxicity likely attributable, as for chemotherapy, to the unselected target cells. In these patients, the mean short duration of the efficacy likely reflects the higher number of mechanisms potentially responsible for the development of resistance (Roskoski 2014, Fey *et al.* 2016, Granata *et al.* 2016). Recently, a plethora of these mechanisms of resistance has been investigated in melanoma skin cancer (Wellbrok & Arozarena 2016). Table 1 summarizes the principal probable reasons of the discrepancy between the advances in biological knowledge and the persistently poor outcomes of advanced breast cancer.

## Advanced breast cancer: prognostic relevance of tumour growth and immune evasion

In advanced breast cancer, the 'driver genes' or recurring 'significantly mutated genes' involved in the 'genetic signatures' that are specific for the molecular subtypes are known, in addition to some principal pathways and molecular cascades that they activate or inhibit (Cancer Genome Atlas Network 2012, Stephens *et al.* 2012). However, it appears that a lot of information regarding the regulation and posttranslational modifications of the altered genes, the multiple signalling cascades they activate, their positive and negative loops and their interconnections is still unknown (Le Romancer *et al.* 2011). In addition, the way in which a single molecular pathway and subcircuit contribute to the final hallmarks is unknown. The complexity of the system suggests that the overall integrated network on which each tumour is based is by far unknown, thereby facilitating the development of resistance to any conventional therapy. Thus, the complexity of the pathobiological model of advanced cancer, which has been uncovering following the progress in genetics and molecular biology, can be compared to the knowledge of the universe following the Hubble advent. This complexity accounts for the relatively poor clinical outcome. In fact, not temporally planned, not appropriately directed and/or not appropriately synergized targeting of one or a few molecular signalling pathways is unlikely to affect the outcome of such complex and

**Table 1** Probable main reasons of the discrepancy between the biological advances in knowledge and persistent relatively poor outcome of advanced breast cancer from therapies.

| System | Problem | Result | References |
|---|---|---|---|
| Tumour | *Spatial heterogeneity:* Many genetic and epigenetic alterations that differ within the same tumour and from one to another. The contribution of the epigenetic alterations is unexpectedly relevant | Any tumour has the own 'genetic signature' that differs from any other | Yachida *et al.* (2010), Verigos & Magklara (2015) |
| | *Temporal heterogeneity:* Genomic instability and biologic plasticity are relevant properties of cancer cells | Any tumour can change its phenotype during progression | Wang *et al.* (2014) |
| Microenvironment | *The stroma contribution:* The cross-talk between stroma and cancer cells | Further increase in the complexity of the overall network of the molecular pathways | Nicolini & Carpi (2009), Hanahan & Weinberg (2011) |
| | The 'contextual signalling': different extracellular signals present in the immediate milieu | Different adaptive responses of cancer cells | Smithson *et al.* (2016) |
| Conventional therapies | Time-limited efficacy | Toxicity, early arising of resistance | Collins (2014) |

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entirely displayed pathological system of any advanced cancer, especially advanced breast cancer. Despite this, we think that the relationship between tumour growth and immune evasion can offer new therapeutic opportunities via an efficacious immune manipulation.

## Tumour growth and prognosis

Several findings have highlighted the clinical relevance of proliferation signalling in breast cancer. In particular, few studies have shown its significant relationship with patient outcome. Luminal A, which is the most common molecular breast cancer subtype with a favourable prognosis, exhibits low expression of cell proliferation-related genes compared with luminal B, which is characterized by a more aggressive phenotype and high expression of these genes (Galanina *et al.* 2011, Eroles *et al.* 2012). A high expression of cell proliferation-related genes is common in basal-like breast cancer (BLBC) or TNBC, associated with the worst prognosis among the different molecular subtypes (Perou *et al.* 2000). A PARADIGM analysis of basal-like vs luminal tumours demonstrated that hyperactivated FOXM1 is a transcriptional driver of this enhanced proliferation signature. Basal-like cancers have 80% of tumour suppressor protein (TP53) mutations; furthermore, the loss of retinoblastoma-associated protein (RB) 1 and BRCA1 genes and high phosphatidylinositol-3 kinase/protein kinase B (AKT) pathway activities are common features of this molecular subtype (Cancer Genome Atlas Network 2012). Moreover, a basal-specific trans-module enriched for transcriptional changes involving cell cycle, DNA damage repair and apoptosis and reflecting the high mitotic index typically associated with basal-like cancers has been described (Curtis *et al.* 2012). In two large-scale studies, a high ratio of the homeobox 13 to IL-17B receptor (IL-17BR) expression correlated with poor clinical outcome in resected node-negative ER-positive breast cancer patients receiving adjuvant tamoxifen. Interestingly, IL-17BR plays a role in recurrences, either by the induction of anti-tumour immunity or by mediating the response to growth factors involved in breast epithelial tumour proliferation (Erlander *et al.* 2005, Goetz *et al.* 2006, 2008). In another study (Paik *et al.* 2004), the expression of 16 cancer-related and five reference genes were used to calculate a recurrence score (RS) for predicting the outcome of tamoxifen-treated, node-negative breast cancer patients. In a multivariate Cox model, the RS was significantly predictive of distant recurrence and overall survival (OS). In this study, the 16 selected genes were grouped on the basis of function, correlated expression or both. Two of the four groups, termed the proliferation and human epidermal growth factor receptor (HER)-2 groups, included five (KI67, STK15, survivin, CCNB1 and MYBL2) and two (GRB7, and Her2) of the 16 selected genes, respectively. Therefore, approximately half of the genes used to calculate the RS were directly related to tumour growth. In a successive investigation, the same RS was prognostic for tamoxifen-treated node-positive breast cancer patients and predicted a significant response to chemotherapy in patients with a high RS (Albain *et al.* 2010).

## Immune signatures and prognosis

Recently, well-described immune signatures have been reported in many studies on gene expression. Specifically, some prognostic immune signatures have been developed for HER-2+ ER-α−, TNBC or BLBC. This is of particular relevance as these breast cancers are among the molecular subtypes that are the most aggressive and resistant to therapy. In the studies on HER-2+ ER-α− breast cancers (Liu *et al.* 2012, 2017), the developed 17-gene immune signature, in addition to the high prognostic value, allowed the identification of patients who would benefit from combination therapy with trastuzumab and immunomodulatory drugs. A biological network-driven gene selection in TNBC (Bonsang-Kitzis *et al.* 2015) identified a stromal six metagene signature named immunity 1, immunity 2, proliferation/DNA damage, androgen receptor-like, Matrix/Invasion 1 and Matrix 2 clusters with the immunity two metagene having a high positive prognostic value. In a study on BLBC (Martínez-Canales *et al.* 2017), 16 genes associated with immune function and upregulated in BLBC compared with their expression in normal breast tissue were linked with improved clinical outcome. In particular, the association of upregulated HLA/T-cell immunoreceptor with IG and ITIM (TIGIT) domains and HLA-C/HLA-F/TIGIT genes showed the most favourable outcome. In other two studies, the immune signature predicted benefit from trastuzumab in adjuvant (Perez *et al.* 2015) or neoadjuvant (Varadan *et al.* 2016) settings. Interestingly, in these and other studies (Levy *et al.* 2016, Heimes *et al.* 2017, Kim *et al.* 2017), when immunological signature was associated with immune function and immune response, it directly correlated with a better clinical outcome. Moreover, epigenetic alterations, in addition to the genetic alterations, of immune genes with prognostic impact have been increasingly reported in different types of cancers, including breast cancer. In an investigational study

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(Xu *et al.* 2016), all 10 B7 family members were amplified in breast cancer. In particular, B7 mRNA levels were upregulated in a cohort of 1098 patients with different types of breast cancer and in 82 patients with TNBC. Promoter methylation analysis showed an epigenetic basis for the deregulation of certain B7 family genes, and only B7-H6 amplification was significantly associated with worse OS. In a further experimental study (Jeschke *et al.* 2017), DNA methylation markers were profiled to identify a methylation of tumour-infiltrating lymphocyte (MeTIL) signature. The MeTIL signature measured TIL distribution in a sensitive way and predicted improved survival and response to chemotherapy in breast cancer better than the histopathological evaluation of TILs or gene expression-based immune markers. Tables 2 and 3 summarize the prognostic role of proliferation and immune signatures.

## Relationships between tumour growth and immune evasion

### Experimental studies

An increasing number of recent experimental studies have reported proliferation and tumour growth to be closely linked to immune evasion in breast cancer. A few of them are briefly described here. In one experimental study, tumour growth was found to be largely COX dependent through immune evasion, thus supporting COX activity as a driver of immune suppression (Zelenay *et al.* 2015). This observation has been confirmed by another experimental investigation, where mammary carcinoma cell-derived COX 2 was found to suppress tumour immune surveillance by enhancing intra-tumoural immune checkpoint activity. In the same study, the examined v-erb-b2 avian erythroblastic leukaemia viral oncogene homolog 2 (ErbB2) transgenic mice that were deficient in mammary epithelial cell COX-2 (COX-2 MEC-KO mice) showed the decreased expression of Ki 67, a proliferation marker, and contained more CD4+ Th cells and CD8+ cytotoxic immune cells compared with wild-type mice, indicating enhanced immune surveillance. Moreover, in ErbB2-transformed mouse breast cancer cells, where lentiviral shRNA delivery was used to knock down COX-2, growth was strongly suppressed (Markosyan *et al.* 2013). The results of another study (Loi *et al.* 2016) suggested that Ras-mitogen-activated protein kinase (MAPK) pathway activation induces immune evasion in TNBC. In particular, genetic or transcriptomic alterations in Ras–MAPK signalling were significantly correlated with lower TILs. Moreover, MEK inhibition upregulated cell surface MHC expression and PD-L1 in TNBC cells both *in vivo* and *in vitro*. In a different study (Zhang *et al.* 2015*a*), hypoxia-inducible factor 1 directly upregulated the transcription of CD47 expression in breast cancer cells, promoting the evasion of phagocytosis by macrophages and the maintenance of CSCs. In another study (Olesch *et al.* 2015), microsomal prostaglandin E synthase (mPGES-1/2) in human and mouse models of breast cancer was shown to favour immune evasion. In addition, mPGES-1 inhibition increased CD80 expression by tumour-associated phagocytes, which triggered cytotoxic T-cell activation and restricted tumour growth. A downregulation of miR-148a, which is closely involved in cancer cell proliferation, has been reported in both ER-positive breast cancer and TNBC. An experimental investigation (Tao *et al.* 2014) has validated the hypothesis that E2 downregulates miR-148a through C protein-coupled oestrogen receptor-1 (GPER) and that E2 also affects the expression of HLA-G, which is an miR-148a target gene. Therefore, a new mechanism based on the ability of oestrogenic GPER signalling to trigger HLA-G expression through the inhibition of miR-148a, which supports immune evasion in breast cancer, has been elucidated. Epidermal growth factor receptor (EGFR) signalling is often dysregulated in TNBC and is also associated with increased glycolysis. A study focused on these aspects (Lim *et al.* 2016) showed that the increased aerobic glycolysis induced by EGFR signalling

**Table 2** Prognostic clinical relevance of proliferation signatures in breast cancer.

| BC subtype/subgroup | Expression | Prognosis | Ref. |
|---|---|---|---|
| Luminal A vs luminal B | Lower in luminal A vs luminal B | Better | Galanina *et al.* (2011), Eroles *et al.* (2012) |
| Basal-like/TNBC | High | Worst | Perou *et al.* (2000), Cancer Genome Atlas Network (2012), Curtis *et al.* (2012) |
| N– ER+ | High HOXB13 to IL-17BR ratio | Poor | Erlander *et al.* (2005), Goetz *et al.* (2006, 2008) |
| TAM treated N– or N+ | High RS with 16 selected genes, 7 of them being proliferation (5) or HER2 (2) related genes | Poor | Paik *et al.* (2004), Albain *et al.* (2010) |

ER+, ER-alpha positive; HOXB13, homeobox13; IL-17BR, interleukin-17 B receptor; N– or N+, axillary lymph-node negative or positive; TAM, tamoxifen; TNBC, triple-negative breast cancer (also see text).

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**Table 3** Immune signatures and prognosis in breast cancer.

| BC subtype/subgroup | Type of immune signature | Prognosis | Ref. |
|---|---|---|---|
| HER2+ | Immune index increase after brief exposure to trastuzumab | Better response (to neoadjuvant CT + H) | Varadan *et al.* (2016) |
| Early HER2+ | 9 or more of 14 immune function gene enriched tumours | Benefit (from adjuvant H) | Perez *et al.* (2015) |
| HER2+ REalpha– | 17-Gene HER2-TIC-enriched signature (HTICS+)* | Benefit (from adjuvant CT + H) | Liu *et al.* (2012, 2017) |
| BLBC | HLA-F/TIGIT and HLA-C/HLA-F/TIGIT upregulated genes | Significantly better DFS and OS | Martínez-Canales *et al.* (2017) |
| TNBC | Weak immunity two metagene expression | Poor DSS | Bonsang-Kitzis *et al.* (2015) |
| TNBC, luminal, HER2+ | High Metil score | Better outcome | Jeschke *et al.* (2017) |

*This signature includes genes related to cell proliferation, immune response and cell migration.
BLBC, basal-like breast cancer; CT, chemotherapy; DSS, disease-specific survival; H, trastuzumab; HLA, human leukocyte antigen; Metil, methylation of tumour-infiltrating lymphocytes; OS, overall survival; RFS, relapse free survival; TIC, tumour-initiating cells; TIGIT, T-cell immune receptor with IG and ITIM domains; TNBC, triple-negative breast cancer (also see text).

in TNBC promotes cell proliferation and tumour growth accompanied by immune escape. In a MMTV-HER2/neu mouse mammary tumour-initiating cells (TICs) model, a 17-gene HER2-TIC-enriched signature (HTICS) predicted the clinical outcome in multiple independent HER2+ cohorts. Four of the eight upregulated genes in HTICS were involved directly in cell cycle progression, DNA replication and mitosis. The upregulation of these genes was concomitant with the downregulation of genes involved in immune response, thus favouring immune evasion (Liu *et al.* 2012).

## Clinical studies in metastatic breast cancer with an undetectable or detectable non-growing tumour burden following conventional therapy

In the last decade, we have reported very promising results (Nicolini & Carpi 2005, Nicolini *et al.* 2005, 2007, 2008) and the possible rationale (Nicolini *et al.* 2006, Nicolini & Carpi 2009) of an open pilot clinical study using a new schedule of conventional anti-oestrogen therapy combined with immune stimulation. We have more times published both results (Nicolini & Carpi 2005, Nicolini *et al.* 2014*a*) and their interpretation (Nicolini *et al.* 2014*b*, 2015, 2016). Progression-free survival (PFS) and median OS times since the diagnosis of distant metastases in 31 endocrine-dependent breast cancer patients were 33 and 94 months, respectively. In 24 of these patients with high levels of hormone dependency (55% of ER-positive progesterone (Pgr)-positive), the median OS was 98.5 months and the delayed median PFS was 45 months compared with those in the remaining seven subjects with lower hormone dependency (20% of ER+ Pgr+), in whom the median OS was 37 months and the median PFS was 20 months. It is noteworthy that 16% of the patients have survived for more than 10 years in complete remission. The following mechanistic interpretation has been proposed: 'within the TME, stromal cells, infiltrating lymphocytes and tumour cells foster tumour growth and immune evasion through a complex network of autocrine and paracrine loops mediated by cytokines and growth factors'. In an anti-oestrogen-responsive metastatic disease, a stable or decreased tumour burden and a lower genetic instability due to quiescent state (G0–G1 state) of tumour cells are also likely to reduce immune evasion by the downregulation of immune escape and immune inhibition. This favours the immune attack stimulated by the sequential administration of IFN-β and IL-2, which, by synergizing with anti-oestrogen therapy, can delay hormone resistance and clinically result into a prolonged response or stable disease. The larger the tumour working portion, the higher its biological aggressiveness. In those with shorter median survival and a likely lower hormone dependence, an expected greater and more aggressive portion of the tumour burden worked with a higher production of cytokines and growth factors. This can explain the earlier occurrence and perhaps more effective immune inhibition during the progression of metastatic disease in those surviving for <5 years. On the other hand, in those surviving for >5 years and with higher levels of hormone dependence, an expected smaller and probably less aggressive portion of the tumour burden worked with lower production of cytokines and growth factors. This may have permitted the immune system to work more effectively for a longer time. Moreover, during the clinical benefit, we showed that 'laboratory evidence of the effect of immunotherapy as well as that hormone resistance occurs at the progression of the disease concomitantly

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with a laboratory pattern compatible with immune inhibition' (Nicolini *et al.* 2007, 2016). We have recently published the last update on the issue (Nicolini *et al.* 2018). In another pilot study, a maintenance immunotherapy with low-dose IL2 and 13-cis retinoic acid was administered to 100 consecutive metastatic breast cancer patients with a clinical benefit (complete response (CR)+ partial response (PR)+ stable disease (SD)) from 6 to 8 courses of induction chemotherapy. There were 68 ER+ and/or Pgr+ patients, all of whom, after the induction of chemotherapy, received endocrine therapy with luteinizing hormone-releasing hormone (LHRH) analogues or letrozole in addition to the maintenance immunotherapy according to whether they were pre- or post-menopausal. In the 100 patients, the median PFS and OS were 37.1 and 57.5 months, respectively. PFS and OS were 44.7 and 64.5, respectively, in the 68 patients with ER+ tumours compared with 32.7 and 51.4, respectively, in the 23 patients with ER− tumours (Recchia *et al.* 2008). In this study, the authors highlighted 'a sustained improvement in lymphocytes, NKs and CD4+/CD8+ ratio with respect to baseline values'. In an earlier pilot study from the same research group, a maintenance immunotherapy with IFN-β, retinyl palmitate and tamoxifen until progression was administered to 23 metastatic breast cancer patients who had achieved a clinical response (11 CR and 12 PR) following six courses of induction and two successive courses of consolidation chemotherapy. All the 23 patients were unresponsive to the hormonal therapy. The PFS and OS were 31.4 and 44 months, respectively, in the 23 responders (CR+PR). The OS was 66 months (with 9-year survival rate of 34%) in the 11 complete responders and 17 months in the non-responders (seven with SD and six with progressive disease) (Recchia *et al.* 1998). The reported median OS in ER+ Her2-negative metastatic breast cancer is 25 months (Savci-Heijink *et al.* 2015) to 30.6 months (Zielinski *et al.* 2016). In these last two pilot studies, the median OS was longer than expected in similar populations and was even longer in the two subsets of ER+ and complete responders (64.5 and 66 months, respectively). However, in these two subsets, the median OS was shorter than just reported (94 months). Indeed, in our pilot study, all the patients were selected because they showed a clinical benefit during anti-oestrogen therapy before receiving the additional immune therapy (Nicolini & Carpi 2005). In the first of the last two pilot studies, all the 68 ER+ patients received maintenance immunotherapy without any previous clinical evaluation of response to the hormone therapy. In the latter, all 23 of the recruited patients were unresponsive to the hormone therapy. In these two last pilot studies, the authors clearly refer to the well-documented immune modulation in addition to the anti-proliferative action by IFNs and retinoids. In particular, the capacity of retinoids to increase the number of IL-2 receptors and peripheral blood lymphoid cells expressing the surface markers of Th cells (Prabhala *et al.* 1991) is mentioned. Moreover, the function of retinoids, which is to facilitate the differentiation of immature myeloid suppressor cells (Gr1+ CD115−), is reported (Huang *et al.* 2006). The biological activity of retinoids is mediated by nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which are ligand-activated transcription factors and are basally present in breast cancer cells. More recently, the relevant role of retinoids for breast cancer chemoprevention and treatment because of their ability to induce cell differentiation and growth suppression (Garattini *et al.* 2014, Seo *et al.* 2015) has been highlighted. Although ER+ breast tumours are also RAR sensitive and are mainly activated by all trans-retinoic acid and 13-cis-retinoic acid (Garattini *et al.* 2014), RXRs are critical for the growth of ER− breast cancer cells (Uray & Brown 2011) and are targeted by a special class of retinoids called rexinoids (Uray & Brown 2011, Seo *et al.* 2015). Table 4 summarizes the data from these three studies. Another study (Greenberg *et al.* 1996) has reported the long-term outcome of 1581 metastatic breast cancer patients from 18 successive front-line trials conducted from 1973 to 1982 at the University of Texas, MD Anderson Cancer Center. All the patients received induction-phase and maintenance chemotherapy that was usually continued for 2 years. The analysis identified 26 (1.6%) patients who were potentially cured among the 1581 evaluated individuals. In fact, they remained in first complete remission after a median duration of 191 months. All 26 of these patients participated along with 263 subjects who had achieved complete remission on anthracycline–cyclophosphamide-based front-line chemotherapy; comparison of the 26 patients with the overall 263 complete responders and total patient populations showed that they had an initially lower tumour burden. In the above-described studies, a longer than expected clinical benefit and OS were observed in patients with or without an immune modulation and/or active immune stimulation following response to the conventional anti-proliferative treatment: anti-oestrogens or conventional cytotoxic chemotherapy. In all of these studies, a low tumour burden was associated with a better clinical outcome; in some of these studies, laboratory data showed that immune therapy stimulated the immune response.

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**Table 4** Clinical outcome in metastatic breast cancer patients treated with hormone therapy and immune manipulation.

| Condition | Recruited patients | | | Hormone therapy and immune manipulation | Studied patients | | Controls | | Reference (*n*) |
|---|---|---|---|---|---|---|---|---|---|
| | *N* | Type of study | Control group | | PFS (month) | OS (month) | PFS (month) | OS (month) | |
| On clinical benefit during tamoxifen as first-line therapy (induction time) | 31 | Pilot Phase II | Historical | Tamoxifen plus interferon-beta and interleukin-2 sequence | 33 | 94 | 13 | 31 | Nicolini & Carpi (2005)Nicolini *et al.* (2014a) |
| Responders (CR+PR) to conventional CT | 100 | Pilot Phase II | Literature data | LHRH analogue (premenopausals) or letrozole (postmenopausals) plus interleukin-2 and retinoic acid | 37.1 | 57.5 | Chung *et al.* (1996), Coates *et al.* (2003), Gennari *et al.* (2006), Alba *et al.* (2007) | | Recchia *et al.* (2008) |
| On clinical benefit following previous conventional CT | 23 | Pilot Phase II | Literature data | Tamoxifen plus interferon-beta and retinyl palmitate | 31.4 | 44 | Sherwin *et al.* (1983), Muss *et al.* (1985), Buzzi *et al.* (1992) | | Recchia *et al.* (1998) |

Clinical benefit (CR+PR+SD).
CR, complete response; CT, chemotherapy; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease.

## Clinical studies in lung, ovarian and colorectal cancers

Here, we summarize clinical studies in advanced cancers other than breast cancer, which contribute to the understanding of the relationships of tumour burden with clinical outcome and likely with immune surveillance (Table 5). The studies can be clustered into two groups. The first group includes studies on a population of patients showing a clinical benefit (CR+PR+SD) following conventional chemotherapy. The other group includes studies on patients with an undetectable residual metastatic disease following radical resection.

## Patients with an undetectable or detectable non-growing metastatic tumour burden following conventional chemotherapy

In this subgroup, three studies conducted by Recchia *et al.* can be included. In two phase II studies on advanced ovarian and advanced non-small-cell lung cancer, IL-2 and 13-cis retinoic acid, respectively, were given as maintenance therapy to patients responsive to and with measurable metastatic disease after conventional chemotherapy. In the advanced ovarian cancer trial, 96% of the patients receiving immune maintenance treatment were responders, 64% of whom showed CR (Recchia *et al.* 2005); in the other study (Recchia *et al.* 2006), 53% of the patients were the responders, only 6% of whom showed CR. In both studies, the remaining patients had SD. The above-described immune maintenance treatment was cyclically self-administered by educated patients and 2 months was considered to represent a single cycle of therapy. The PFS and OS were the secondary end points for comparison with historical data from controls who were chosen to perfectly match the patients in the study group. The PFS and OS curves showed a statistically significant improvement in IL-2/13-cis retinoic acid-treated patients. For example, in the advanced ovarian cancer trial, the median PFS and OS were 50.5 and 102.5 months, respectively, in the study group compared with 15.5 and 29.6 months, respectively, in the controls. In the advanced non-small-cell lung cancer trial, the median PFS and OS were 16.5 and 23.4 months, respectively, in the study group compared with 8.4 and 11.8 months, respectively, in the controls. Another investigation using the same schedule of immunotherapy was conducted by the same research group in metastatic colorectal cancer patients who had a clinical benefit (CR+PR+SD) from induction chemotherapy (Recchia *et al.* 2006). In this study,

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**Table 5** Clinical relevance of tumour burden for an active or probably induced immune manipulation in cancer other than breast.

| | | | | **Clinical outcome** (month) | | |
|---|---|---|---|---|---|---|
| **Tumour burden** | **Cancer type** | **Pts, *n* (%)** | **Immune-manipulation** | PFS/DFS (median; month) | OS (median; month) | **References** |
| Undetectable (CR or radical surgery) or detectable (PR, SD) not growing disease following conventional CT | Ovary* | CR, 88 (64)<br>PR, 14 (32)<br>SD, 2 (4) | IL-2 plus 13-cis-RA | 50.5 vs 15.5 (controls)<br>$P<0.00012$*** | All 102.5 vs 29.6 (controls)<br>$P<0.0001$*** | Recchia *et al.* (2005) |
| | Lung* (non-small) | CR, 3 (6)<br>PR, 17 (34)<br>SD, 18 (36) | IL-2 plus 13-cis-RA | 16.5 vs 8.4 (controls)<br>$P<0.0003$*** | All 23.4 vs 11.8 (controls)<br>$P<0.0007$*** | Recchia *et al.* (2006) |
| | Colon* | CR, 10 (25)<br>PR, 11 (27)<br>SD, 19 (48) | IL-2 plus 13-cis-RA | 27.8 vs 12.5 (controls)<br>$P<0.0001$*** | All 52.9 vs 20.2 (controls)<br>$P<0.0001$*** | Recchia *et al.* (2006) |
| | GI* (mixed) | 19 (100) | Few cycles of additional conventional CT regularly given | 80.4% 5-year vs 31.8% expected | 87.1% 5-year vs 40.1 expected | Nicolini *et al.* (2010) |
| Undetectable (m. r. d.) following radical surgery with or without adjuvant CT | Colorectal | 1001 (100) | No active immune-manipulation | NA | 22% 10-year | Fong *et al.* (1999) |
| | | 173 (100) | | NA | 27% 10-year | Scheele *et al.* (1990) |
| | | 86 with CT (100)<br>85 without CT (100) | | DFS and 5-year DFS<br>24.4 and 33.5% vs 17.6 and 26.5% (controls)<br>$P=0.028$ | OS and 5-year OS<br>62.1 and 51.1% vs 46.4 and 41.1% (controls)<br>$P=0.13$ | Portier *et al.* (2006) |
| | | 138 with CT (100)<br>140 without CT (100) | | PFS and 5-year DFS<br>27.9 and 36.7% vs 18.8 and 27.7% (controls)<br>$P=0.058$ | OS and 5-year OS<br>62.2 and 52.8% vs 47.3 and 39.6% (controls)<br>$P=0.095$ | Mitry *et al.* (2008) |
| | | 6254** (100) (meta-analysis) | | 5-year DFS ranging from 13% to 46% | | Liu *et al.* (2016) |

*pilot study; **neoadjuvant CT prior to hepatic resection; ***log-rank test.
13-cis-RA, 13-cis-retinoic acid; CR, complete response; CT, chemotherapy; DFS, disease-free survival; GI, gastrointestinal; HT, hormone therapy; IL-2, interleukin-2; m. r. d., minimal residual disease; NA, not available; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease (also see text).

25% were complete responders. After a median follow-up of 36 months, the median PFS was 27.8 months in the 40 recruited patients of the study group and 12.5 months in the 80 controls. The median OS was 52.9 in the study group and 20.2 in the controls. In all the three pilot trials, the number of total lymphocytes, NK cells, CD4+/CD8+ ratio and VEGF were determined in the peripheral blood of both the studied patients and controls. At baseline in all three studies, none of the evaluated immunological parameters in the two treatment groups (studied patients and controls) were statistically different. In the ovarian cancer study, a progressive increase in the lymphocyte count in IL-2-treated patients and a progressive decrease in the controls was observed, and the difference after 1 year became statistically significant in both the lung and colorectal cancer studies ($P<0.01$ and $P<0.0001$, respectively). Similarly, the NK cells increased in IL-2-treated patients compared with that in the controls, and after 1 and 2 years (ovarian cancer study) or 1 year (lung and colorectal cancer studies) the difference became statistically significant ($P=0.03$ and $P=0.0007$ for ovarian cancer; $P=0.04$ and $P<0.0001$ for lung and colorectal cancer, respectively). Again, in all the three studies, the CD4+/CD8+ ratio values increased in IL2-treated patients

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and decreased in controls; after 1 and 2 years (ovarian cancer study) or 1 year (lung and colorectal cancer studies), the difference became statistically significant within the same group before and after maintenance immunotherapy and between them (IL-2 treated vs controls). Finally, in all the three studies, the baseline VEGF values of IL-2-treated patients showed a statistically significant decrease after 1 year (lung cancer study, $P=0.0002$). In the other two studies, the same significant decrease was maintained at 1 and 2 years (ovarian cancer study, $P<0.0049$; colorectal cancer study, $P<0.0001$). Unlike in responding patients, in those progressing during conventional chemotherapy, immunotherapy is more likely unsuccessful. In fact, in one of two recently published clinical trials conducted in advanced non-small-cell lung cancer using immune checkpoint (CTL antigen 4, PD-1 and PD L1) inhibitors, a 30% increase in PFS (3.5 vs 2.8 months) has been reported compared with the conventional chemotherapy (Brahmer *et al.* 2015); in the other, without PFS improvement, there was a 30% increase in the OS (12.2 vs 9.4 months) (Borghaei *et al.* 2015). In the same target population of advanced non-small-cell lung cancer in the previously mentioned experimental trial conducted by Recchia *et al.* (2006), the significant PFS and OS improvement observed in patients treated with IL-2 and 13-cis retinoic acid maintenance therapy was 100% (8.4 vs 16.5 months and 11.8 vs 23.4 months, respectively) compared with that in the controls. However, in the pilot study by Recchia *et al.*, the patients were recruited in clinical benefit, whereas in those conducted with the new immunological drugs; the patients were recruited when they were in progression following previous conventional therapy. Thus, this different condition at the time of recruitment may have substantially affected the outcome. Overall, these findings suggest that an appropriate immune maintenance therapy can significantly improve the clinical outcome of patients with a detectable non-growing metastatic tumour burden. This improvement seems to be correlated with the proportion of complete responders to conventional chemotherapy, i.e., with the tumour burden.

## Patients with gastrointestinal (GI) cancer and an undetectable residual metastatic disease following radical surgery

In a pilot study conducted by a group (Nicolini *et al.* 2010) in patients with GI cancers who were apparently disease free after primary surgery and had a high risk of relapse due to residual undetectable metastases, an almost double 5-year disease-free survival (DFS; 80.4%) and OS (87.1%) was reported compared with the expected. In this study, starting from the first year after conventional adjuvant chemotherapy till the fifth year, patients received 2–3 cycles of additional adjuvant chemotherapy using infusional 5-fluorouracil (FU) plus leucovorin. Moreover, it has been reported that 22–27% of colorectal cancer patients are 10-year survivors following radical resection of synchronous or metachronous liver metastases without any adjuvant chemotherapy (Scheele *et al.* 1990, Fong *et al.* 1999). Conventional adjuvant or neoadjuvant chemotherapy commonly with infusional 5-FU plus leucovorin significantly increased the 5-year DFS rate from 27–42% to 37–46% (Portier *et al.* 2006, Mitry *et al.* 2008, Liu *et al.* 2016). It is noteworthy that the extension of primary tumour and liver recurrences were among the most significant predictors of worse prognosis in all the trials and that only about 20% of colorectal cancer patients are chemosensitive to infusional 5-FU plus leucovorin (Nicolini *et al.* 1998). In patients with an undetectable minimal residual disease, an induced or spontaneous recovery of the immune surveillance can be predicted. An undetectable or detectable non-growing tumour burden following conventional chemotherapy and an undetectable minimal residual metastatic disease following radical surgery are more suitable conditions for immune manipulation. The former condition, which likely occurred in our study and other mentioned pilot studies, benefited from an actively induced immune stimulation or immune maintenance therapy that improved the clinical outcome. Interestingly, in this condition, a prolonged 'resting state' (G0-G1 state) was likely due to the hormone therapy allowing more efficacious immune manipulation and better clinical outcome. In addition to the already mentioned immune-modulating properties of retinoids and preclinical evidence (Moon *et al.* 1983, Sporn & Roberts 1983) of their key role in controlling normal cellular proliferation and differentiation, it is well known that IL-2 is the principal growth factor for lymphocytes (Nicolini *et al.* 2006). In addition, there is clinical evidence (Lippman *et al.* 1992, Frasci *et al.* 1993, The Nordic Myeloma Study Group 1996) supporting the efficacy of IFN therapy combined with conventional chemotherapy or retinoids in the settings of locally advanced or minimal residual disease of breast and other types of cancer. In the latter case, the reduction by surgical removal and/or a conventional anti-proliferative therapy of a previously well-detectable and extended cancer to minimally undetectable residual metastatic disease could have favoured the spontaneous recovery of the immune surveillance. This maintained

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a small fraction of these patients in the disease-free and potentially healthy state. Lower residual metastatic tumour burden, chemosensitivity and other not yet well-understood reasons likely led to the selection of this small fraction of patients. This suggests that tumour burden and proliferation directly correlate with immune evasion and with the complexity of the activated network that sustains each cancer. The concept of a link between tumour burden and immune tolerance is also gaining acceptance within the scientific community (Cimino-Mathews *et al.* 2015, Clifton *et al.* 2015, Migali *et al.* 2016).

## A refined pathobiological model and a novel therapeutic strategy

In prolonged 'resting state' (G0-G1 state) non-growing condition during anti-oestrogen therapy (Osborne 1994, Wolf *et al.* 1994, Doisneau-Sixou *et al.* 2003) or in the 'minimal residual metastatic disease' molecular pathways promoting invasion and diffusion, angiogenesis and reprogramming energy and metabolism are likely downregulated, without clinical relevance. This is consistent with the finding that the rate of definitely cured patients after adjuvant therapy and/or primary operation is inversely correlated with tumour size at diagnosis. Accordingly, angiogenesis and metastatic processes are strictly linked to the progression of cancer and the shift from an oxidative to glycolytic metabolism, mainly through the 'Warburg effect', is favoured by hypoxia concomitant with tumour growth. Therefore, following these and the previously reported data and concepts about the relationship between tumour growth and immune evasion, we propose to refine the pathobiological model by Hanahan and Weinberg, as shown in Fig. 1. By this model, long-term active anti-proliferative therapies and minimal residual disease are the conditions mostly favouring an efficacious immune manipulation.

## Endocrine-dependent cancers

Recently, the genetic background of proliferation-promoting and -inhibiting action of oestrogens and anti-oestrogens, respectively, has been evaluated in-depth, and the immunosuppressive function of sex hormones has been largely documented. These findings are summarized below.

### ER-α-regulated genes in MCF-7 human breast cancer cell lines increase tumour growth

ER-α is a transcription factor that regulates many genes that play important roles in physiology and are also involved in the development and progression of breast cancer. MCF-7 cells have shown that although ER-α interacts with thousands of genomic regions, E2-responsive genes range from 100 to 1500 (Charpentier *et al.* 2000,

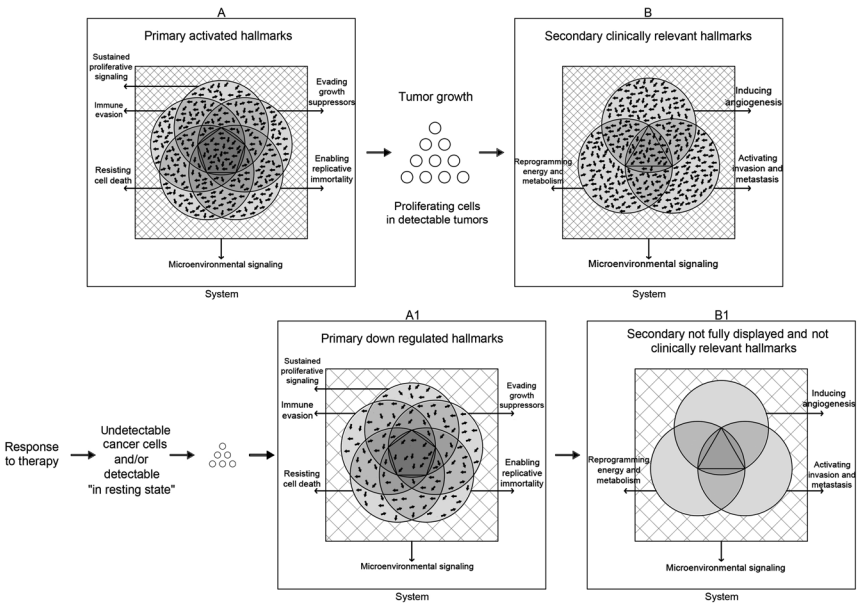


**Figure 1**
A refined pathobiological model for advanced breast cancer, relationship of tumour growth with immune evasion. (A and B) Any sphere represents an activated subcircuit sustained by a pathological molecular network (short arrows) converging to the hallmarks (long arrows). The different activated pathological molecular networks at least in part overlap each other (in shadow areas); the square represents the signalling (grid) from a supportive tumour microenvironment cross-talking with the subcircuits; both pentagon and triangle represent active genomic instability and inflammation (additional integrated hallmarks). (A1 and B1) Any sphere represent a downregulated subcircuit; the different pathological molecular networks at least in part overlap each other (in shadow areas); the square represents the downregulated cross-talk of the microenvironment (grid) with the subcircuits; both pentagon and triangle represent the downregulated genomic instability and inflammation. Reprinted from *Cell*, vol **144**, D Hanahan & RA Weinberg, Hallmarks of cancer: the next generation, pages 646-674, copyright (2011), with permission from Elsevier.

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Coser *et al.* 2003, Frasor *et al.* 2003, Carroll *et al.* 2006, Kininis *et al.* 2007, Lin *et al.* 2007). In previous study, Frasor *et al.* (2003) summarized their findings and stated that 'many genes whose expression is altered by E2 are associated with specific cell signalling pathways and regulatory factor receptor loops. These include a general upregulation of positive proliferation regulators and the downregulation of negative proliferation regulators, which together may contribute to the overall stimulation of proliferation and suppression of apoptosis'. Welboren used ChIP-Seq to map ER-α-binding sites and to profile changes in RNA polymerase II occupancy in MCF-7 cells in response to E2, tamoxifen or fulvestrant (Welboren *et al.* 2009). Overall, 1256 genes and five different clusters of genes were identified. In particular, many genes encoded proteins binding the nucleus and RNA binding the mitochondrion. Moreover, E2 induced the downregulation of pro-apoptotic genes Bad, Bak, Bik and cyclin A and of genes involved in cell cycle arrest or proliferation, such as cyclin G2, a negative regulator of the cell cycle that maintains cells in a quiescent state. Cyclin D1 and IGF-binding protein 4 were the other regulated genes governing cell proliferation and growth. In another more recent study (Hah *et al.* 2011), the authors demonstrated 'a potent effect of E2 signalling on the protein biosynthetic machinery, which fits well with the known mitogenic effects of E2 on MCF-7 cells' and highlighted that 'E2 signalling has strong, immediate and likely direct effects on transcription by all three RNA polymerases'. In a research study (Kininis *et al.* 2007) aimed at exploring the global mechanisms of oestrogen-regulated transcription, the authors reported that 'many of these direct E2 target genes exhibit interesting modes of regulation and biological activities, some of which may be relevant to onset and proliferation of breast cancers (e.g. UGT2B15, CYP1B1 and PRUSE)'. In a review article following the above report, the same authors (Hah & Kraus 2014) concluded that 'the most immediate effects of oestrogen signalling on the genome results in the regulation of mRNAs encoding proteins involved in transcription, nucleic acid metabolism, and G protein-coupled and cell surface signalling. Thus, oestrogen signalling propagates the hormone-dependent transcriptional response, leading to secondary and sustained effects. Over the long term, oestrogen signalling upregulates the protein biosynthesis machinery. This is likely how the oestrogen signalling pathway prepares the cell for translation of the mRNAs that are newly synthesized in response to oestrogen signalling. The immediate and sustained effects of oestrogen signalling underlie the mitogenic effects of oestrogen signalling in breast cancers' (Fig. 2).

## Tamoxifen inhibits most ER-α-mediated proliferation genes

In a study conducted by Frasor *et al.* (2004), the effects of different SERMs were investigated in MCF-7 cells. Based on the results, the authors stated that 'it is apparent that many of the genes on which the SERMs act as antagonists could affect cell proliferation' and that 'their ability to block the E2 stimulation of cell proliferation suggests that the genes they antagonize are those that are essential for the stimulatory effect of E2 on cell proliferation'. They concluded that 'it is of interest that several of these genes have potential tumour suppressor or anti-proliferative activities in breast cancer cells and could contribute to the beneficial effects of transhydroxytamoxifen'. A successive investigation by the same author focused on genes not or minimally regulated by E2 and preferentially regulated by tamoxifen in ER-α-positive MCF-7 human breast cancer cells (Frasor *et al.* 2006). Among the 64 genes preferentially regulated by tamoxifen (50 upregulated

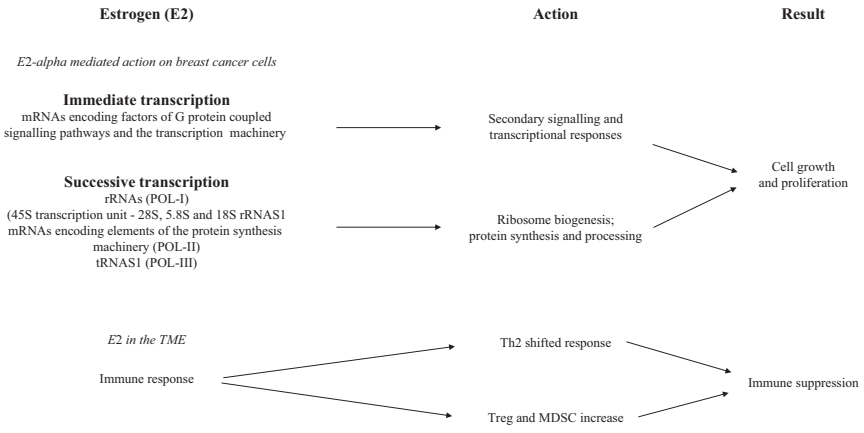


**Figure 2**
Effect of ER on breast cancer cells and tumour microenvironment (TME). MDSC, myeloid-derived suppressor cell (also see text).

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and 14 downregulated) were PKIA, an inhibitor of cyclic AMP-dependent protein kinase A activity; PTPRG, a receptor type protein tyrosine phosphatase; and SOCS1, an inhibitor of JAK/STAT signalling; PTPRG and SOCS1 have potential tumour suppressor roles. All have the capacity 'to alter different cellular signalling pathways and, thus, responsiveness of breast cancer cells to other hormones, growth factors or cytokines. In addition, IEX1 has been shown to have growth-inhibitory effect suggestive of a beneficial effect of tamoxifen'. Two tamoxifen upregulated genes, namely YWHAZ and LOC441453, showed significant association with disease recurrence. YWHAZ likely plays a relevant role in insulin receptor and EGFR signalling and in cell cycle regulation. Overall, the findings reported in the two studies by Frasor were confirmed in another study by Welboren *et al.* (2009). In this successive research, most of the E2-upregulated genes were antagonized by tamoxifen, which mainly showed agonistic behaviour on E2-downregulated genes. In addition, a group of genes was the only target of tamoxifen. Table 6 summarizes the gene ontology of tamoxifen ER-α-mediated genes assessed in some principal studies. The studies performed recently and mentioned above show that genes that are mostly affected by oestrogens and anti-oestrogens are proliferation genes.

### Sex hormones and the immune response

Reportedly, testosterone has a general suppressive effect on the immune function (Roved *et al.* 2017). In particular, testosterone has dampening effects on many innate immune cells (monocytes, macrophages, dendritic cells (DCs), granulocytes, NK cells, platelets and endothelial cells) and in DCs, it may also downregulate the expression of MHC class II receptors and co-stimulatory molecules (Koh *et al.* 2009, Hepworth *et al.* 2010). Regarding adaptive immunity, its action on type 1 (Th1) response is uncertain, whereas a significant decrease in type 2 and Th17-induced immune responses due to the suppression of functions associated with Th2 and Th17 differentiation has been reported (Hepworth *et al.* 2010, Yao *et al.* 2003, Kissick *et al.* 2014). Th1 cells mostly activate macrophages and CD8+ CTLs, whereas Th2 cells mainly stimulate B cells to produce antibodies and Th17 cells to produce inflammatory cytokines (particularly IL-17 and IL-22) (Murphy & Weaver 2016). Regarding estrogens and adaptive immunity, Foo *et al.* in a meta-analysis of 38 studies (Foo *et al.* 2017) found that oestrogens induce positive effects on humoral immunity but a significantly decreased effect on cell-mediated immunity. In particular, oestrogens shift adaptive immune responses in favour of type 2 immune responses (Faas *et al.* 2000), whereas type 1 and Th-17 responses are suppressed (Wang *et al.* 2009, Tyagi *et al.* 2012, Chen *et al.* 2015*a*,*b*). Data suggesting the promoting or inhibiting role of Th-17 and IL-17 on tumorigenesis have been reported. Some findings (Alinejad *et al.* 2017) have suggested that through activation of the ERK1/ERK2, NF-kb and BCL-2 pathways, the IL-17B/IL-17RB system promotes inflammation, breast cancer progression as well as resistance to chemotherapy drugs (Alinejad *et al.* 2016). Conversely, some findings have shown that IL-17 significantly induce MDSC differentiation, inhibit their proliferation and trigger apoptosis through the JAK/STAT3 pathway *in vitro* (Ma *et al.* 2018), whereas other findings (Benchetrit *et al.* 2002, Kryczek *et al.* 2009) have supported an anti-tumour effect against certain tumours. A recent review (Rothenberger *et al.* 2018) focusing on the role of the oestrogen pathway in the TME has confirmed that oestrogen promotes immune suppression through the modulation of pro-tumour responses independent of direct activity on tumour cells (Fig. 2). In particular, data have suggested that oestrogens in the TME 'shift the balance in favour of Th2 responses, production of tumour-promoting cytokines (IL-6, IL-4, TNF-α and IL-17A) and M2 TAM infiltration compared to the Th1 responses, associated Th1 cytokines (IL-12 and IFNγ) and M1 TAM infiltration'. Moreover, oestrogens are likely 'to promote tumour immune evasion through the proliferation of Treg cell and MDSC populations, augmented tumour cell PD-L1 expression and inhibition of CD8+ T-cell- and NK cell-induced apoptosis. In addition, CAFs may support the TME by providing paracrine sources of oestrogens and IL-6'. All these studies have clarified that many genes and, consequently, multiple molecular pathways whether directly or indirectly involved in breast cancer proliferation are induced by oestrogens and inhibited by anti-oestrogens. This, in addition to the relevant role of anti-oestrogens in inhibiting an immune-suppressive TME, promoted by oestrogens, makes them ideal candidates in the battle against cancer either alone or in combination with other drugs (Rothenberger *et al.* 2018).

### Locally advanced or metastatic disease

A successful immune manipulation is more probable in patients with metastatic disease in clinical benefit during anti-oestrogen therapy. In fact, anti-oestrogens, by directly acting on multiple genes or indirectly inhibiting proliferation, promote a prolonged 'resting state' (G0-G1 state) (Osborne 1994, Wolf *et al.* 1994,

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**Table 6** Principal reported transhydroxytamoxifen (TOT) or tamoxifen (TAM) actions on ER-alpha mediated E2-regulated or E2-not regulated genes.

| Experimental model | SERMs | ER-alpha mediated genes (*n*) | | | | Some main tumour growth related genes | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | E2 regulated (*n*) | | Not or minimally E2 regulated (*n*) | | Gene | Cluster | Gene | Cluster | |
| ER+ MCF7 cells | TOT | Full or partial antagonist Cluster A | Partial agonist/ antagonist or full agonist Cluster B | Cluster C1 | Cluster C2 | AREG (E2 up) | A | RAB30 (Ral, TOT or ICI up) | C1 | Frasor *et al.* (2004) |
| | | | | | | SDF1 (E2 up) | A | TPM1 (Ral, TOT or ICI up) | C1 | |
| | | | | | | TGF-beta2 (E2 down) | A | IER3* (E2 down) | A | |
| | | 18 E2 up 16 E2 down | 18 E2 up 16 E2 down | 24 TOT up | 51 TOT down | INHBB (E2 down) | A | CCNA2 (Ral, TOT or ICI down) | C2 | |
| | | | | | | RB1CC1 (Ral, TOT or ICI up) | C1 | CDKN2C (Ral, TOT or ICI down) | C2 | |
| | | | | | | CDK8 (Ral, TOT or ICI down) | C2 | | | |
| | TAM | NE | NE | 50 TAM up | 14 TAM down | PTPRG (TAM up) SOCS1 (TAM up) IEX1* (TAM up) | | RAB30 (TAM up) YWHAZ (TAM up) | | Frasor *et al.* (2006) |
| | | 548 E2 up TAM down | – | – | | GO: protein binding nucleus, RNA binding, mithocondrion (protein synthesis) | | | | Welboren *et al.* (2009) |
| | | 172 E2 up TAM down | – | – | | GO: cell proliferation, regulation of growth and others | | | | |
| | | 179 E2 down Not or partially TAM downregulated | – | – | | GO: positive regulation of apoptosis and cell proliferation, cell cycle arrest | | | | |

*IER3 and IEX1: the same gene has been reported in the two A and C different categories (Frasor *et al.* 2004, 2006) (also see text).
AREG, amphiregulin; CCNA2, cyclinA2; CDK8, cyclin dependent kinase 8; CDKN2C, cyclin dependent kinase inhibitor 2C; E2, TAM, TOT up, estradiol, tamoxifen, or transhydroxytamoxifen upregulated; E2, TAM, TOT down, estradiol, tamoxifen, or transhydroxytamoxifen downregulated; GO, gene ontology; ICI, ICI182.780; IER3, immediate early response 3; INHBB, inhibitin beta B; PTPRG, protein tyrosine phosphatase receptor type G; RAB30, member of RAS oncogene family; Ral, raloxifene; RB1CC1, RB1-inducible coiled-coil1; SDF1, stromal cell-derived factor-1 (also known as chemokine ligand 12); SERMs, selective estrogen receptor modulators; SOCS1, suppressor of cytokine signalling 1; TGFbeta2, transforming growth factor beta2; TPM1, tropomyosin 1(alpha); YWHAZ, 14-3-3zeta.

Doisneau-Sixou *et al.* 2003) concomitant with a non-growing tumour (clinical benefit) or a decrease in tumour burden to 'minimal residual metastatic disease' (CR). The probable concomitant downregulation of the multiple mechanisms responsible for immune tolerance permit an active immune modulation/stimulation, which, as reported above in the work of our group and other authors, significantly prolong the PFS and/or OS. Thus, first, in ER-positive metastatic breast cancers, the same schedules of immune-modulatory/stimulatory treatments combined with anti-oestrogens should be validated in large prospective randomized trials. Moreover, in the same population of ER-positive patients but with locally advanced cancer, they should be investigated as adjuvant treatments. The combination of hormone therapy with immunotherapy could be considered for the same duration for which conventional anti-oestrogens are currently recommended (5–7 years). It can be inferred that by replacing conventional anti-oestrogens with anti-androgens, the same schedules of hormone immune therapies proposed in endocrine-dependent breast cancer could be evaluated in metastatic and locally advanced hormone-dependent prostate cancers.

## Endocrine-independent cancers at high risk of relapse

### Locally advanced

In endocrine-dependent cancers with *de novo* or acquired hormone resistance and other types of high-risk endocrine-independent solid cancers (gastrointestinal, lung and ovary), it is currently unproven and unlikely to obtain a prolonged 'resting state' of resistant cancer cells. In these patients, conventional anti-proliferative drugs and/or therapeutic interventions (surgical and/or radiological) should aim to decrease the tumour burden as much as possible. In patients with locally advanced cancer and postoperative minimally residual disease, the analysis of proliferation markers Ki67 and p120 have shown that approximately 16% of disseminated tumour cells are in an active cell cycle, whereas the majority remain arrested in G0 phase (Pantel *et al.* 1993). Tumour dormancy and the concomitant multi-drug resistance in the chemosensitivity test can explain the frequent inefficacy of adjuvant chemotherapy. In dormant cells, a gradual proliferation can be triggered by the changes in their microenvironment and/or the acquisition of additional genetic 'hits' (Köstler *et al.* 2000). Thus, in these patients, in the initial 6–8 months after conventional adjuvant chemotherapy and/or radical resection, regular administration of a few cycles (3–4) of anti-proliferative drugs with concomitant immune-modulating properties, such as taxanes or antimetabolites (5-FU, capecitabine), at low doses every 8–12 months for a few years can be considered (Nicolini *et al.* 2010). In fact, it is likely that at least a few months are needed for residual resistant cancer cells to grow and activate the multiple concomitant mechanisms necessary to mount a significant immune tolerance in these patients. Thus, the main aim of this additional adjuvant chemotherapy is to interrupt the probable 'works in progress'. In fact, this procedure is expected to gradually switch off the mechanisms triggering the proliferation of residual resistant cancer cells and the concomitant immune evasion. Paclitaxel administered once a week for

**Table 7** Proposal of a novel therapeutic strategy for advanced breast and other cancers treatment.

| Setting | Target population | Therapeutical interventions | Main aim | Cancer type | Reference |
|---|---|---|---|---|---|
| Adjuvant at high risk or metastatic | Endocrine responsive* | Large prospective randomized multicenter trials with hormone-immunotherapy | Significant PFS and/or OS increase | Breast and Prostate | Nicolini *et al.* (2014*a*) |
| Adjuvant at high risk | Endocrine resistant/independent likely with m. r. d. | 3–4** cycles of taxanes or antimetabolites (5-FU, capecitabine) with or without partially synergizing immune drugs regularly given every 8–12 months, for 5 years | Significant delay or decrease of the recurrence rate | Breast and other solid tumours | Recchia *et al.* (1998, 2006, 2007), Nicolini *et al.* (2010) |
| Metastatic in CR, PR or SD following conventional CT | Endocrine resistant/independent with detectable metastatic disease | Immunomodulatory and/or immunostimulating drugs | To delay metastatic progression | | |

*In prostate cancer, anti-androgens replace anti-estrogens; **taxanes or antimetabolites (5-FU, capecitabine) should be chosen in relation to cancer type (breast and other solid cancers) according with current therapeutic recommendations.
CR, complete response; m. r. d., minimal residual disease; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease.

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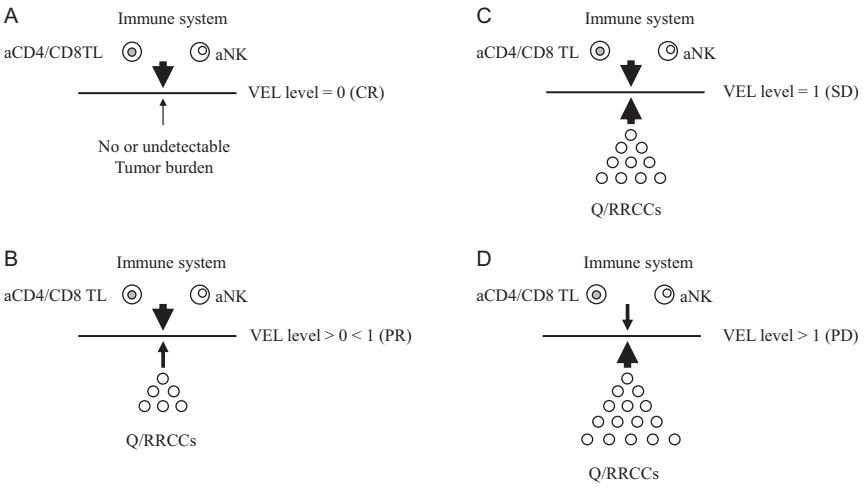


**Figure 3**
Different levels of a virtual equilibrium line (VEL) in tumours with undetectable burden or with prolonged 'resting state' of residual cancer cells following conventional therapy. (A) CR: immune system strongly prevails and eliminates all or most Q/RRCCs; (B) PR: immune system prevails and eliminates any new formed cancer cell but only some Q/RRCCs; (C) SD: a perfect equilibrium occurs and immune system eliminates any new formed cancer cell but no Q/RRCCs; (D) PD: immune system eliminates less new cancer cells than they are formed by Q/RRCCs (also see text). aCD4/CD8TL, activated CD4/CD8 T lymphocyte; aNK, activated NK cell; Q/RRCCs, quiescent/resistant residual cancer cells; VEL, virtual equilibrium line.

2 weeks every 3 weeks or 5-FU infusion administered for 5 days every 28 days (1 cycle) blocks MDSC expansion in addition to the well-known anti-proliferative effect (Sevko *et al.* 2012). Low dose of paclitaxel also has been reported to simultaneously inhibit Treg cells (Sevko *et al.* 2012) and exert immune-stimulatory effects on DCs at ultra-low doses (Shurin *et al.* 2009). On the other hand, 5-FU can counter one or more ways of immune attack evasion by tumour cells, such as downregulation of MHC class I antigens, loss of Fas expression and shedding of tumour antigens (Khallouf *et al.* 2012). Potentially synergizing immune drugs, such as IFNs, IL-2 and retinoids, can be included. These therapeutic schedules could significantly delay or decrease recurrences, as shown in GI cancers (Nicolini *et al.* 2010).

### Metastasis in clinical benefit during conventional therapy

In cases of resistant/non-endocrine-dependent breast and other types of solid cancers in clinical benefit (CR+PR+SD) following conventional therapy, immune maintenance therapy (immunomodulatory/stimulating drugs) can be attempted to delay the regrowth of tumour. In these cases, provided that immune tolerance is directly correlated with tumour burden (Migali *et al.* 2016), more therapeutic effect is expected when immune manipulation follows CR or PR rather than SD (Recchia *et al.* 2005, 2006, 2007). Table 7 summarizes these proposals.

## A virtual equilibrium line between tumour burden and the immune system

In patients with undetectable or prolonged quiescent/non-growing state of residual cancer cells, the efficacy of the immune response (cure of the disease or delay of the occurrence of resistance to therapy) can be determined by the level at which a virtual equilibrium line is positioned by the opposite activities of cancer and immune cells. In fact, under these conditions, the tumour burden is balanced between the actions from residual resistant cancer cells and the spontaneously or actively modulated/stimulated immune response. The pressure exerted by the resistant proliferating cancer cells can be successfully or not counterbalanced by the immune response. When a CR occurs, it is likely that the spontaneously induced or actively stimulated immune system prevails so that most or all of the residual resistant cancer cells are eliminated and the equilibrium line is positioned at level 0. When a PR occurs, the immune system is likely to inhibit the formation of or eliminate any new cancer cells and only some of the residual resistant cancer cells; therefore, the line is positioned at level $>0<1$. When a SD occurs, a perfect equilibrium is established and the immune system inhibits the formation of or eliminates any new cancer cells, but not residual resistant cancer cells, and the line remains at level 1. In case of a disease progression, the activated immune system likely eliminates new cancer cells less than they are derived from the residual resistant cancer cells, and the equilibrium line is positioned at level $>1$. Any factor that affects the immune activation, the amount of residual resistant cancer cells and their phenotype can change the position of the equilibrium line (Fig. 3).

## Conclusions

In this article, the recent major advances acquired in molecular biology of cancer were the premise to discuss the apparent discrepancy in the poor prognosis of advanced

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breast cancer. In reality, it has become increasingly clear that advanced cancer, especially advanced breast cancer, is an entirely displayed pathological system that is much more complex than previously considered. Thus, the understanding of cancer is a work in continuous progress. General rules governing the entirely displayed pathological cancer system, if existing, should be identified. Experimental and clinical studies on breast cancer and other types of cancer support the notion of a close relationship between tumour growth and immune evasion. Based on these findings, we propose a novel therapeutic strategy and a refined pathobiological model for advanced breast and other solid cancers. The novel therapeutic strategy is based on therapeutically induced conditions (undetectable tumour burden or a prolonged tumour 'resting state'), which enable an efficacious immune response in advanced breast and other types of solid cancers.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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