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EDITORIAL

Impact of treatment schedule on the efficacy of cytostatic and immunostimulatory agents

Giulia Petroni[a] and Lorenzo Galluzzi[a,b,c,d,e]

[a]Department of Radiation Oncology, Weill Cornell Medical College, New York, USA; [b]Sandra and Edward Meyer Cancer Center, New York, USA; [c]Caryl and Israel Englander Institute for Precision Medicine, New York, USA; [d]Department of Dermatology, Yale School of Medicine, New Haven, Connecticut, USA; [e]Université de Paris, Paris, France

ABSTRACT

Radiation therapy (RT) and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors mediate poorly overlapping cytostatic and immunostimulatory effects, suggesting that combinatorial regimens may enable supra-additive tumor control. Our preclinical findings demonstrate that administration schedule stands out as a major determinant of efficacy when RT and CDK4/6 inhibitors are combined for breast cancer therapy.

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Estrogen receptor (ER)$^+$ breast cancer (BC) is the most common BC subtype, accounting for approximately 80% of all BC cases and the majority (>60%) of BC-related deaths in the US.[1] For decades, patients with resectable ER$^+$ BC have been treated with surgery followed by endocrine therapy (ET), optionally combined with radiotherapy (in the case of conservative surgery) and/or chemotherapy (depending on risk for relapse).[2] Conversely, metastatic ER$^+$ BC has classically been managed with ET or neoadjuvant chemotherapy, often based on rather immunostimulatory agents like taxanes or anthracyclines.[3,4] Recently, the development of agents specific for cyclin-dependent kinase 4/6 (CDK4/6), which are key drivers of cell cycle progression in ER$^+$ BC cells,[5] has considerably altered this approach.[6] Indeed, the addition of CDK4/6 inhibitors (*i.e.* palbociclib, abemaciclib, and ribociclib) to ET has been associated with a robust survival advantage over ET alone in women with metastatic ER$^+$ BC,[7,8] rapidly resulting in the implementation of CDK4/6 inhibitors plus ET as first-line therapeutic strategy for these patients. However, >50% of women with ER$^+$ BC receiving CDK4/6 inhibitors plus ET experience disease progression within 24 months from treatment initiation,[7,8] calling for the identification of novel strategies to maximize the therapeutic efficacy CDK4/6 inhibitors and further delay disease progression in this patient population.

Accumulating preclinical and clinical evidence suggests that the therapeutic synergy between CDK4/6 inhibitors and ET involves (at least in part) their ability to promote anticancer immunity. Indeed, letrozole-based ET has been associated with limited BC infiltration by immunosuppressive CD4$^+$CD25$^+$FOXP3$^+$ regulatory T (T_{REG}) cells.[9] Moreover, palbociclib and other CDK4/6 inhibitors not only resemble letrozole in its ability to deplete T_{REG} cells, but also promote MHC Class I expression on malignant cells (*de facto* rendering them more visible to the immune system)[10] and favor the secretion of type III interferon (IFN), which is a potent immunostimulant.[11,12] This suggests that immunological mechanisms including the establishment of an immunosuppressive tumor microenvironment may support clinical resistance to CDK4/6 inhibitors plus ET, and that additional immunostimulatory agents may be harnessed to circumvent it. In this context, radiation therapy (RT) stands out as an ideal partner for CDK4/6 inhibition as: (1) both RT and CDK4/6 inhibition have cytostatic effects, but operate at different phases of the cell cycle (*i.e.* G_2/M and G_1, respectively);[13,14] (2) RT mediates pronounced immunostimulatory effects via pathways that are largely independent of those elicited by CDK4/6 inhibitors, including type I IFN secretion;[15,16] and (3) the safety profile of RT is well established,[17] and preliminary retrospective data from a cohort of metastatic ER$^+$ BC patients suggest that the administration of CDK4/6 inhibitors plus RT is reasonably well tolerated.[18]

Based on these premises, we set to investigate the potential synergy between RT and the prototypic CDK4/6 inhibitor palbociclib, harnessing human ER$^+$ and ER$^-$ BC cell lines and three different immunocompetent mouse models of BC. In particular, we aimed at identifying an optimal dosing and administration schedule with the ultimate objective to inform the design of clinical trials testing the combination of RT and palbociclib in patients with oligometastatic HR$^+$ BC in the context of letrozole-based ET. Upon confirming that palbociclib and RT employed as standalone therapeutic agents arrest the proliferation of human ER$^+$ BC MCF7 cells in G_1 and G_2/M, respectively, we combined these agents according to the

CONTACT Giulia Petroni giuliapetroni@gmail.com Department of Radiation Oncology, Weill Cornell Medical College, New York, USA; Lorenzo Galluzzi deadoc80@gmail.com Weill Cornell Medical College, Stich Radiation Oncology, 525 East 68th Street, Box # 169, New York, NY 10065 USA

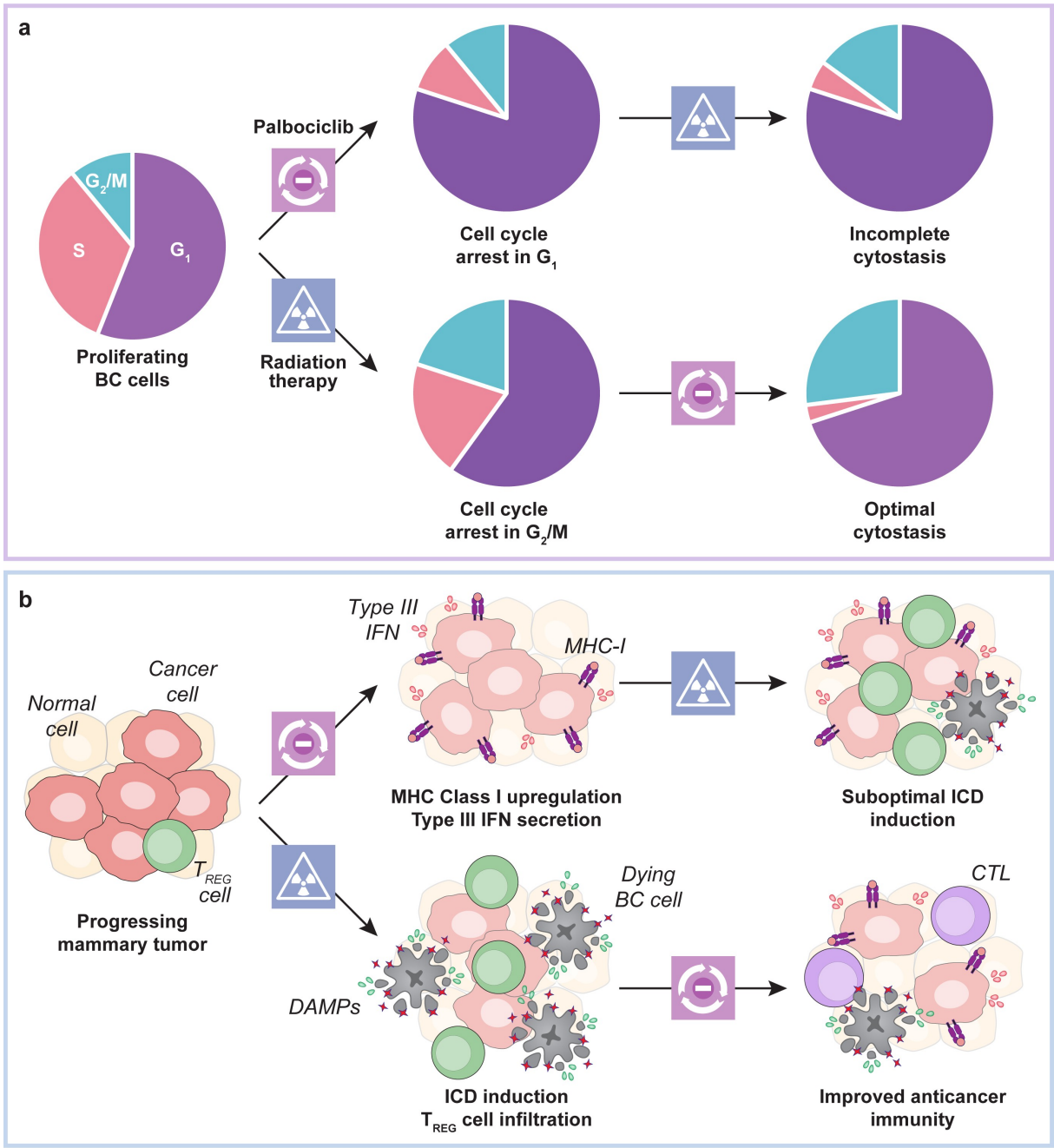

Figure 1

Figure 1. Impact of treatment schedule on the efficacy of radiation therapy combined with CDK4/6 inhibitors. a. Palbociclib administration arrests breast cancer (BC) cells in the G_1 phase of the cell cycle, resulting in the generation of at least some radioresistance upon the depletion of cells in G_2/M (a). While palbociclib may *per se* mediate some immunostimulatory effects, including MHC Class I upregulation and the secretion of type III interferon (IFN), the virtually pure cytostasis imposed by palbociclib may prevent the initiation of optimal anticancer immunity upon irradiation, which is at least partially dependent on immunogenic cell death (ICD) (b). Radiation therapy (RT) blocks BC cells in G_2/M (a), a process that (1) at least in some cells is leaky, meaning that such cells can complete mitosis and resume cycling at G_1, and (2) is associated with at least some degree of ICD-driven immunostimulation coupled to compensatory tumor infiltration by regulatory T (T_{REG}) cells. In this context, the administration of palbociclibafter RT not only mediates superior cytostasis as it arrests BC cells potentially escaping the RT-induced arrest in G_2/M, but may also boost anticancer immunity elicited by ICD as a consequence of T_{REG} cell depletion (b). CTL, cytotoxic T lymphocyte; DAMP, damage-associated molecular pattern.

following treatment schedules: (1) P→RT, RT delivered after palbociclib, or (2) RT→P, palbociclib delivered after RT. We found that RT→P mediates superior short-term (3 days) and long-term (14 days) cytostatic effects in MCF7 cells as compared to P→RT, reflecting the near-to-complete inhibition of cell proliferation. Similar data were obtained in human triple-negative breast cancer (TNBC) MDA-MB-231 cells (which at odds with MCF7 bear *TP53* mutations and hence

are expected to enable limited cooperativity between RT and palbociclib).[19,20]

We next evaluated the interactions between palbociclib and focal RT in (1) immunocompetent BALB/c mice bearing subcutaneous, syngeneic p53-incompetent TS/A mammary adenocarcinomas (a widely employed model of ER^+ BC) or 4T1 mammary carcinomas (a widely employed model of metastatic TNBC), or (2) an endogenous model of mammary carcinogenesis driven by a synthetic progestin, i.e. medroxyprogesterone acetate (MPA) and a polycyclic aromatic hydrocarbon, *i.e.* 7,12-dimethylbenz[a]anthracene (DMBA), in immunocompetent C57BL/6J mice. The latter is a privileged platform for translational studies as it recapitulates key immunobiological features of luminal B ER^+ BC in women, including reduced infiltration by immune cells, limited sensitivity to immunotherapy and (most often) wild-type *TP53* status.[21,22] In line with our *in vitro* data, RT→P also demonstrated superior therapeutic efficacy as compared to P→RT *in vivo*, irrespective of p53 competence. Indeed, RT→P was associated with (1) superior local disease control in all models, (2) extended overall survival in TS/A-bearing mice, and (3) limited metastatic dissemination to the lungs in 4T1-bearing mice.[19]

Altogether, our findings support the contention that RT may be harnessed to improve the therapeutic benefits of CDK4/6 inhibitors in patients with metastatic ER^+ BC, potentially linked to the initiation of superior tumor-targeting immune responses. Irrespective of this latter possibility (which we are actively investigating), our data strongly suggest that RT should be delivered before, not after, palbociclib to achieve superior disease control. These findings are in line not only with previous results from human glioblastoma and atypical-teratoid rhabdoid tumor (ATRT) cell lines, cultured *in vitro* and xenografted in immunodeficient mice,[23] but also with the notion that RT is preferentially active in the G_2/M phase of the cell cycle, and hence that palbociclib may limit the fraction of cells responding to RT if administered beforehand. Consistent with this, it has recently been shown that CDK4/6 inhibitors should be delivered after, not before, DNA-damaging chemotherapeutics (which preferentially target cells in S and G_2/M) to achieve an optimal control of cultured pancreatic ductal adenocarcinoma (PDAC) cells and an immunocompetent model of PDAC driven by *Kras* and *Cdkn2a* mutations.[24] Although also in this setting the contribution of the host immune system to treatment efficacy was not mechanistically dissected, various DNA-damaging chemotherapeutics resemble RT in promoting a variant of cell death that elicits robust immunostimulatory effects that can be accompanied by compensatory tumor infiltration by T_{REG} cells,[3,16,25] and hence may set the stage for optimal immunostimulation by CDK4/6 inhibitors. Further supporting this possibility, multiple CDK4/6 inhibitors have been shown to cooperate with immunostimulatory targeted anticancer agents including MEK and PI3K inhibitors in preclinical models of lung and pancreatic cancer.[26]

Our findings inspired the design of a Phase II clinical trial (CIMER, NCT04563507) that randomizes patients with newly diagnosed oligometastatic ER^+ BC (up to 5 metastases) to either palbociclib plus letrozole or palbociclib plus letrozole preceded by stereotactic body RT to each metastatic lesion. As the CIMER protocol involves the collection of tumor biopsies at baseline and at progression, it will be interesting not only to see whether the addition of RT improves disease control by palbociclib plus letrozole, but also to investigate the immunological correlates of progression in both treatment arms. Irrespective of these impending studies, data from us and others provide solid preclinical grounds to harnessing immunostimulatory regimens such as RT as tools for improving the clinical efficacy of CDK4/6 inhibitors in patients with metastatic ER^+ BC, as they highlight treatment schedule as a potential key determinant of therapeutic success (Figure 1).

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ORCID

Lorenzo Galluzzi http://orcid.org/0000-0003-2257-8500

Competing interests

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