International Journal of Cardiology 292 (2019) 171–179

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Cardiotoxicity and pro-inflammatory effects of the immune checkpoint inhibitor Pembrolizumab associated to Trastuzumab

V. Quagliariello [a,1], M. Passariello [b,1], C. Coppola [a], D. Rea [d], A. Barbieri [d], M. Scherillo [e], M.G. Monti [f], R.V. Iaffaioli [g], M. De Laurentiis [h], P.A. Ascierto [i], G. Botti [j], C. De Lorenzo [b,c,*], N. Maurea [a,*]

[a] Division of Cardiology, Istituto Nazionale Tumori- IRCCS- Fondazione G. Pascale, Napoli, Italy
[b] CEINGE – Biotecnologie Avanzate S.C.a.R.L., Naples, Italy
[c] Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Napoli, Italy
[d] Animal Facility, Istituto Nazionale Tumori- IRCCS- Fondazione G. Pascale, Napoli, Italy
[e] Azienda ospedaliera San Pio, Cardiologia Interventistica ed UTIC, Azienda Ospedaliera "G.Rummo" di Benevento, Napoli, Italy
[f] Department of Translational Medical Sciences, University Federico II, Naples, Italy
[g] Association for Multidisciplinary Studies in Oncology and Mediterranean Diet, Piazza Nicola Amore, Naples, Italy
[h] Breast Unit, Istituto Nazionale Tumori- IRCCS- Fondazione G. Pascale, Napoli, Italy
[i] Melanoma, Cancer Immunotherapy and Development Therapeutics Unit, Istituto Nazionale Tumori- IRCCS- Fondazione G. Pascale, Napoli, Italy
[j] Scientific Direction, Istituto Nazionale Tumori- IRCCS- Fondazione G. Pascale, Napoli, Italy

ARTICLE INFO

Article history:
Received 7 January 2019
Received in revised form 19 April 2019
Accepted 13 May 2019
Available online 17 May 2019

Keywords:
Immunotherapy
Cardiotoxicity
Inflammation
Interleukins
Cardio-oncology

ABSTRACT

Background: The immunotherapy has revolutionized the world of oncology in the last decades with considerable advantages in terms of overall survival in cancer patients. The association of Pembrolizumab and Trastuzumab was recently proposed in clinical trials for the treatment of Trastuzumab-resistant advanced HER2-positive breast cancer. Although immunotherapies are frequently associated with a wide spectrum of immune-related adverse events, the cardiac toxicity has not been properly studied.

Purpose: We studied, for the first time, the putative cardiotoxic and pro-inflammatory effects of Pembrolizumab associated to Trastuzumab.

Methods: Cell viability, intracellular calcium quantification and pro-inflammatory studies (analyses of the production of Interleukin 1β, 6 and 8, the expression of NF-kB and Leukotriene B4) were performed in human fetal cardiomyocytes. Preclinical studies were also performed in C57BL6 mice by analyzing fibrosis and inflammation in heart tissues.

Results: The combination of Pembrolizumab and Trastuzumab leads to an increase of the intracellular calcium overload (of 3 times compared to untreated cells) and to a reduction of the cardiomyocytes viability (of 65 and 20–25%, compared to untreated and Pembrolizumab or Trastuzumab treated cells, respectively) indicating cardiotoxic effects. Notably, combination therapy increases the inflammation of cardiomyocytes by enhancing the expression of NF-kB and Interleukins. Moreover, in preclinical models, the association of Pembrolizumab and Trastuzumab increases the Interleukins expression of 40–50% compared to the single treatments; the expression of NF-kB and Leukotriene B4 was also increased.

Conclusion: Pembrolizumab associated to Trastuzumab leads to strong cardiac pro-inflammatory effects mediated by overexpression of NF-kB and Leukotriene B4 related pathways.

1. Introduction

Immune-checkpoint blocking antibodies including anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and anti-Programmed cell death protein 1 (PD-1) can induce tumor responses in melanoma, non-small cell lung cancer (NSCLC), renal cell cancer (RCC), breast cancer and Hodgkin disease [1]. The anti-CTLA-4 monoclonal antibody Ipilimumab and the anti-PD-1 monoclonal antibodies Nivolumab and Pembrolizumab (PEM) have been approved by regulatory agencies in several countries for the treatment of metastatic melanoma and are associated with response rates ranging from 10 to 15% [2], 31–44% [3] and 33–38% [4], respectively. Antibodies against immune checkpoints can also be combined to achieve additive or synergistic activity, potentially translating into a better efficacy. An indicative example is provided by the finding of increased efficacy of Ipilimumab and Nivolumab

* Corresponding authors: C. De Lorenzo, Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Napoli, NA, Italy; N. Maurea, Division of Cardiology, Istituto Nazionale Tumori-IRCCS- Fondazione G. Pascale, Napoli, Italy.
E-mail addresses: cladelor@unina.it (C. De Lorenzo), n.maurea@istitutotumori.na.it (N. Maurea).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijcard.2019.05.028
0167-5273/

combinatorial treatment for metastatic melanoma [5] compared to monotherapies.

The revolution of immunotherapy in the oncology world, however, also includes several side effects that have been recently identified and called immune-related adverse events (irAEs) involving pneumonitis, hepatitis, colitis and the endocrine alterations [6]. For example, 64–80% of patients treated with Ipilimumab recorded irAEs (20% of grade 3 or 4), which reaches 79% of patients treated with PEM [7] (13% with grade 3–4 toxicity). The combined treatment of Ipilimumab and Nivolumab leads to a 96% offside effects compared to placebo groups (55% of which with grade 3–4 toxicity) [8].

In addition, the association of anti PD-1 and anti CTLA-4 antibodies increases the prevalence of myocarditis cases; notably, patients treated with immunotherapies have a 4-fold increased risk of developing major adverse cardiac events, defined as the composite of cardiovascular death, cardiogenic shock, cardiac arrest, and hemodynamically significant complete heart block [9]. Moreover, patients treated with Ipilimumab experienced pericarditis and cardiomyopathies and in cases of treatments with PEM, significant increases of acute heart failure and myocarditis have been observed [10]. The Receptor tyrosine-protein kinase ErbB-2, also called ErbB2/Her2, is overexpressed in many tumors like the breast one and has been an attractive target for immunotherapy in recent years [11]. Trastuzumab (TRA) was the first humanized monoclonal antibody to become a standard of care for metastatic ErbB2-positive breast cancer [12].

Despite the success of this antibody for breast cancer, its clinical efficacy is still limited by resistance and cardiotoxicity events [13–15]. The randomized clinical trial described by Slamon and colleagues [16] showed that 27% of cancer patients treated with TRA and anthracyclines experienced cardiac dysfunctions and heart failures. A recent clinical study called “HERceptin Adjuvant trial” described the incidence of cardiotoxicity in cancer patients treated with TRA monotherapy of 4.4 and 7.25% of the patients experiencing secondary cardiac events (class I or II toxicity) after 1 and 2 years of treatment, respectively [17].

Retrospective studies have recently demonstrated an increased incidence of cardiomyopathy and/or heart failure in patients treated with TRA and these effects can persist for many years after the conclusion of the therapy [18]. Recently, our group has conducted studies on the early detection strategies, pathophysiology and management of the TRA-related left ventricular dysfunction in breast cancer patients [19,20].

A recent phase Ib/II interventional clinical study, called PANACEA trial, demonstrated that the association therapy of PEM and TRA leads to clinical benefits in breast cancer patients [21]. Nevertheless, possible additive cardiotoxic and pro-inflammatory effects on the heart tissue of this association therapy remain substantially unknown. Therefore, we aimed to study the mechanism of the cardiotoxicity related to the association of TRA and PEM both in cellular end preclinical models, with particular attention on the analysis of pro-inflammatory interleukins (Interleukins 1-β, 8 and 6), Leukotriene B4 and p65/NF-kB expression.

2. Methods and materials

2.1. Cell viability

To test the effects of PEM and TRA combinatorial treatments on co-cultures of tumor cells or human fetal cardiomyocytes (HFC) with lymphocytes, the cells were plated in 96-well flat-bottom plates at the density of $1.5 \cdot 10^4$ cells/well for 16 h. Human Peripheral Blood Mononuclear Cells (hPBMCs) from healthy donors were added at effector:target ratio 5:1 in the absence or presence of mAbs, used alone or in combination (both at 200 nM), and incubated for 24 h at 37 °C. Controls included target cells incubated in the absence of effector cells or in the presence of the antibodies, used alone. After treatments, lymphocytes were removed and adherent cells were washed and counted by the trypan blue exclusion test. Cell survival was expressed as percent of viable cells tested with drugs compared to the untreated ones, used as a negative control. The images of the cells untreated or treated with each compound or with their combinations were acquired by a Leica Microsystems integrated microscope (DFC320).

2.2. Effects of the novel combination on the production of Interleukin 2 by hPBMCs

The levels of Interleukin 2 (IL-2) in the supernatants of HFC cells, co-cultured with human lymphocytes and incubated or not with TRA, PEM or their combination, were measured by ELISA method (from DuoSet ELISA, R&D Systems), accordingly to the manufacturer's recommendations. Concentration values were reported as the mean of at least three determinations.

2.3. Intracellular calcium assay

Intracellular calcium in HFC cells was quantified with the fluorescence dye Fluo-3 AM, following the manufacturer's protocol [22]. Briefly, HFC were untreated or treated with TRA or PEM or TRA/PEM in combination as described in paragraph 2.1. After incubation, HFC cells were loaded with 5 μM Fluo-3 AM at 37 °C for 30 min in the dark, and then washed three times with PBS to remove excess of dye. The fluorescence intensity of Fluo-3 chelated with calcium was recorded on a microplate spectrofluorometer (xMark Microplate, Spectrofluorometer Biorad, Milan, Italy) at excitation and emission wavelengths of 488 and 525 nm, respectively.

2.4. p65/NF-kB expression studies

Cardiomyocytes were untreated or treated with TRA or PEM or TRA/PEM in combination as described in Section 2.1; after treatments, cellular extracts were analyzed by using the TransAM NF-κB p65 transcription factor assay kit (Active Motif, Carlsbad, CA), according to the manufacturer's recommendations. NF-κB complexes were captured by binding to a consensus 5′-GGGACTTTCC-3′ oligonucleotide immobilized on a 96-well plate. Bound NF-κB was quantified by incubating with anti-p65 primary antibody followed by horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and spectrophotometric detection at a wavelength of 450 nm by using a microplate spectrofluorometer. Data were expressed as the percentage of p65/NF-κB DNA binding relative to control (untreated) cells.

2.5. Pro-inflammatory interleukins quantification

The expression of pro-inflammatory interleukins, such as Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Interleukin-1β (IL-1 β) in human fetal cardiomyocytes was evaluated with ELISA method, as described elsewhere [22,23]. Briefly, cells were grown and treated as described in paragraph 2.1. Subsequently, culture supernatants were collected, centrifuged to pellet any detached cells, and measured by using IL-1β, IL-6 and IL-8 ELISA kits according to the manufacturer's instructions (Sigma Aldrich, Milan, Italy). The sensitivity of this method was below 10 (pg/ml), and the assay accurately detected cytokines in the range of 1–32,000 pg/ml.

2.6. Animals and experimental design

Preclinical studies were performed following the same procedures described in our previous published cardiological studies [24,25]. Briefly, twenty-four female C57Bl/6 mice were purchased by Harlan, San Pietro al Natisone (Italy) housed (6 per cage) and maintained on a 12 h light-12 h dark cycle (lights on at 7.00 am) in a temperature-controlled room (22 ± 2 °C) with food and water ad libitum at all times. After a period in quarantine, mice were randomized for weight and enrolled in four treatment groups (6/each group). Mice were

intraperitoneally administrated with PEM (anti PD-1; Merck; Whitehouse Station, NJ, USA) at 10 mg/kg for the first dose, followed by 5 mg/kg dose every 5 days until the study end point, according to literature [26]; in another group, mice were intraperitoneally administrated with TRA (Roche, Switzerland) at dose 10 mg/kg/day, according to literature [27]; in another group mice were intraperitoneally administrated with both drugs in combination. Control mice (Sham) were given the same volume of saline solution for three weeks, as well as in all treatment groups. Notably, we decided to use the PEM doses considering other literature sources [26] showing anticancer activities in patient-derived xenograft tumors, called the Onco-HuNSG model, that is the most promising preclinical investigation model aimed to study the anticancer efficacy of immunotherapies. The experimental procedures were performed in compliance with European Directive 63/2010/EU and Italian Law (DL 26/2014, authorized by the Minister of Health, Italy).

2.7. Inflammation studies in cardiac tissues

After the treatments described in paragraph 2.6, the animals were sacrificed by cervical dislocation after anesthesia with tilotamine (0.09 mg/g), zolazepam (0.09 mg/g) and 0.01% atropine (0.04 mL/g) and the total heart was weighted and processed to inflammation studies described herein. The heart tissues were snap-frozen using dry ice until later use for tissue homogenization, which was carried out in 0.1 M phosphate buffered saline (pH 7.4) containing 1% TritonX-100 and protease inhibitor cocktail and processed using high intensity ultrasonic liquid processor. The homogenates were centrifuged at 4 °C and supernatants were used for determination of tissue markers. ELISAs for mouse IL-1β, IL-8 and IL-6 as well as for Leukotriene B4 and p65/NF-Kb in heart tissue extracts were performed by using commercially available kits for mouse following the same procedures described in cellular studies [28] and according to the manufacturer's instructions. Results were expressed as pg of interleukin/mg of heart tissue.

2.8. Cardiac fibrosis

For ex vivo analyses, hearts were excised and fixed in 10% neutral buffered solution, then the myocardial tissue was paraffin-embedded for morphometry. Six μm thick cross sections were deparaffinized and stained with hematoxylin–eosin for general morphology and with Picrosirius red (Carlo Erba Laboratories, Milan, Italy) to detect collagen fibers. Fibrotic areas were observed both in white light and in polarized light, where it was possible to distinguish between type 1 (red-yellowish) and Type 3 (green) collagen fibers. Slides were observed with a Nikon Eclipse E1000 equipped with a polarized set and the software NIS ELEMENTS BRV (Nikon Instruments, Melville, NY, USA).

2.9. Statistical analysis

Values are indicated as mean ± standard deviation (SD). Two-way ANOVA and Bonferroni post-hoc analysis were used to examine the significance of differences among groups (Graph and Prism 5.0; GraphPad Software, Inc., La Jolla, CA, USA). A probability value with $p \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Cellular anticancer efficacy and cardiotoxic effects of Trastuzumab and Pembrolizumab alone or in combination

It has been clinically demonstrated that the association of PEM and TRA leads to a better anticancer efficacy compared to monotherapies [29]. As shown in Fig. 1, A, co-incubation of PEM and TRA with mammary tumor cells leads to a greater anticancer efficacy compared to monotherapies and these effects are more significant in the case of co-culture of cancer cells with lymphocytes (Fig. 1, A) indicating a possible interaction between these cells during exposure to PEM and TRA. Moreover, we investigated the putative side toxic effects of TRA in combination with PEM on HFC cells. As shown in Fig. 1, B, both drugs significantly affected the viability of HFC indicating cardiotoxic effects. The strongest cytotoxic effects were observed when the two antibodies were used in combination, as this led to an inhibition of about 60% of cardiac cell survival, when the mAbs were tested in combination in the presence of lymphocytes.

Since immunomodulatory antibodies, such as PEM, are able to improve T cell effector functions [30] we tested whether the combination of TRA and PEM on HFC cells induce T cell activation by IL-2 cytokine secretion. As shown in Fig. 1, C PEM and TRA slightly stimulated the secretion of IL-2 by hPBMCs, however their co-incubation increased the IL-2 release in a more significant manner. These results are in agreement with the cardiotoxic data, indicating that TRA and PEM in combination are more cardiotoxic compared to the monotherapies.

3.2. Effects of the combination therapy on the intracellular calcium overload in cardiomyocytes

To determine the intracellular calcium level in HFC cells during treatments we used the fluorescence probe Fluo-3 AM as reported elsewhere [22]. Treatment with TRA increased intracellular calcium levels in cardiomyocytes compared to control cells ($p < 0.001$) (Fig. 2, A). The co-incubation with lymphocytes slightly increases the intracellular calcium overload in cardiomyocytes, in both single and combinatorial treatments with the drugs under test. Incubation with TRA and PEM increases intracellular calcium accumulation of 2 and 1.4 fold, respectively, compared to untreated cells ($p < 0.001$ for both). The PEM / TRA combination therapy triplicates the accumulation of intracellular calcium, compared to control cells ($p < 0.001$).

3.3. Effects of the combinatorial treatment on the p65/NF-kB activation in cardiomyocytes

In order to investigate whether PEM and TRA affects the NFκB activation, which is critical for its pro-inflammatory transcriptional activity, the DNA binding activity of NF-κB was analyzed by ELISA assay. As shown in Fig. 2, B, co-incubation with lymphocytes increases of about 20% the activation of p65/NF-kB ($p < 0.05$, compared to only cardiomyocytes); treatment with PEM and TRA leads to a 70% and 90.5% of p65/NF-kB activation, respectively, compared to untreated cells ($p < 0.001$ for both). Also in this case, co-incubation with PEM/TRA has additive pro-inflammatory effects with an increase of 111.1% compared to control cells ($p < 0.001$) and of 11% compared to only TRA-treated cells ($p < 0.05$).

3.4. Inflammation studies

As shown in Fig. 3, A, B and C, the co-culture with lymphocytes slightly increases the concentration of pro-inflammatory interleukins confirming the molecular interactions between these cells. Incubation with PEM increases of 33.7 (Fig. 3, A) and 64.2% (Fig. 3, C) the concentration of IL-8 and IL-1β, respectively, compared to control, without any significant effects on the IL-6 production (Fig. 3, B). Incubation with TRA leads to an increase of IL-8 (Fig. 3, A), IL-6 (Fig. 3, B) and IL-1β (Fig. 3, C) production of 32, 39.1 and 91.7% respectively, compared to untreated cardiomyocytes co-incubated with lymphocytes. Interestingly, the combination therapy has synergistic pro-inflammatory effects increasing of 84, 45.6 and 101% the concentration of IL-8 (Fig. 3, A). IL-6 (Fig. 3, B) and IL-1β (Fig. 3, C), respectively, compared to control cells. Preclinical studies aimed to corroborate the pro-inflammatory effects on TRA and PEM in heart tissues. As shown Fig. 3, D, PEM and TRA increased the inflammation cardiac tissues.

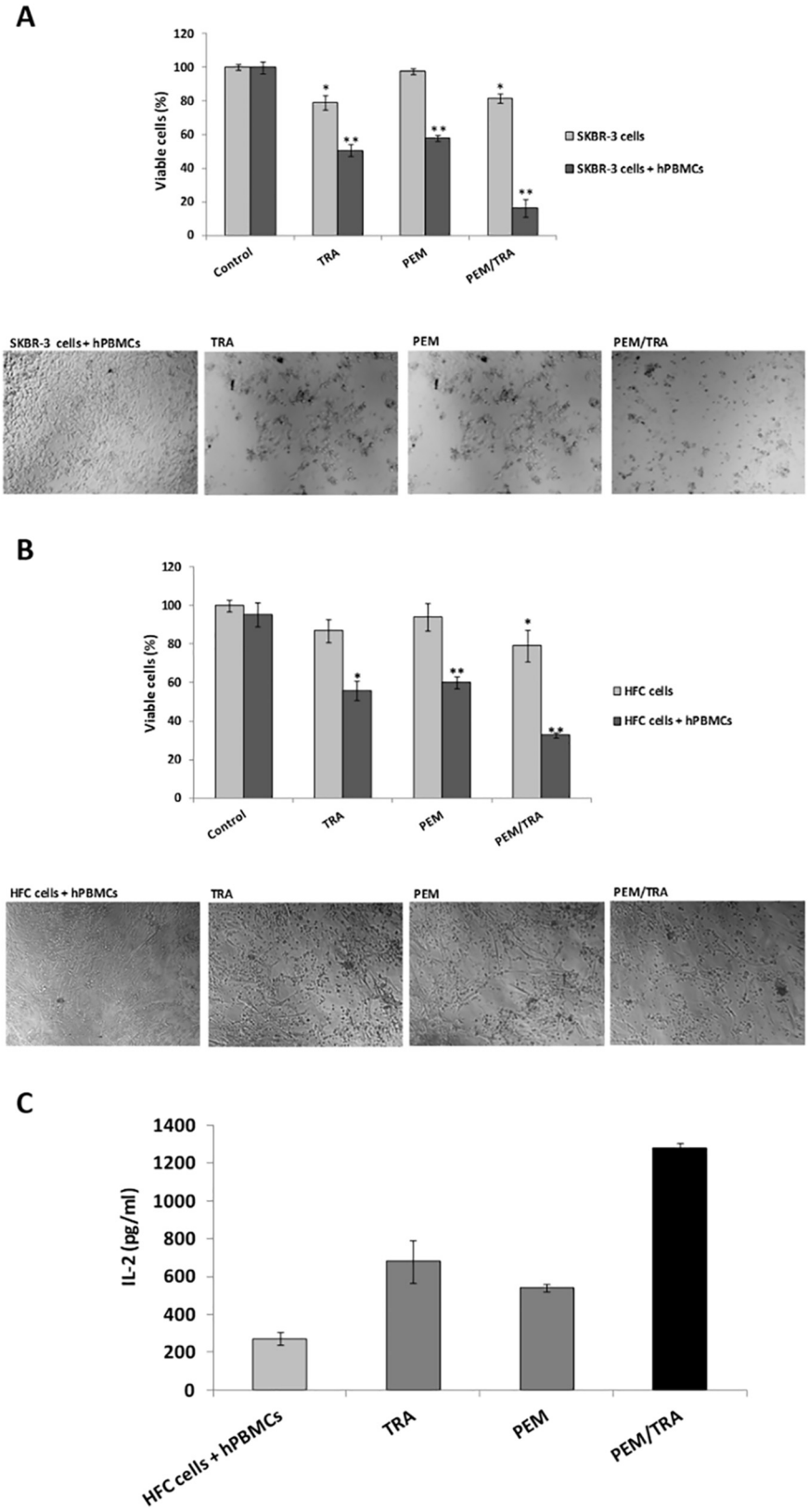

Fig. 1. Effects of combinatorial treatment (Trastuzumab/Pembrolizumab) on co-cultures of SKBR-3 cancer cells and hPBMCs (A) and on co-cultures of HFC cells and hPBMCs (B). A: SKBR-3 mammary cancer cells were cultured in the absence (light grey bars) or in the presence of lymphocytes (dark grey bars) and treated for 24 h with TRA or PEM mAbs used alone, or in combination, at the indicated concentrations (upper panel). SKBR-3 cell survival is expressed as percentage of viable treated cells with respect to untreated cells. Images of SKBR-3 cells treated with each mAb or with the combination of the antibodies tested at the concentration of 200 nM in the presence of lymphocytes (lower panel) removed before the image acquisition. B: HFC cells were co-cultured in the absence (light grey bars) or in the presence of lymphocytes (dark grey bars) and treated for 24 h with TRA or PEM used alone, or in combination, at the indicated concentrations. Cell survival is expressed as percent of viable treated cells with respect to untreated cells. Images of HFC treated with each mAb or with the combination of the antibodies tested at the concentration of 200 nM in the presence of lymphocytes. C: Effects of combinatorial treatment (TRA/PEM) on secretion of IL-2 by hPBMCs co-cultured with HFC cells (lower panel). The untreated cells in the presence of lymphocytes were used as a negative control. The values were reported as the mean of at least three determinations obtained in three independent experiments. Error bars depicted means ± SD. p values for the indicated mAbs relative to control are: $^{*}p < 0{,}05$, $^{**}p < 0.01$.

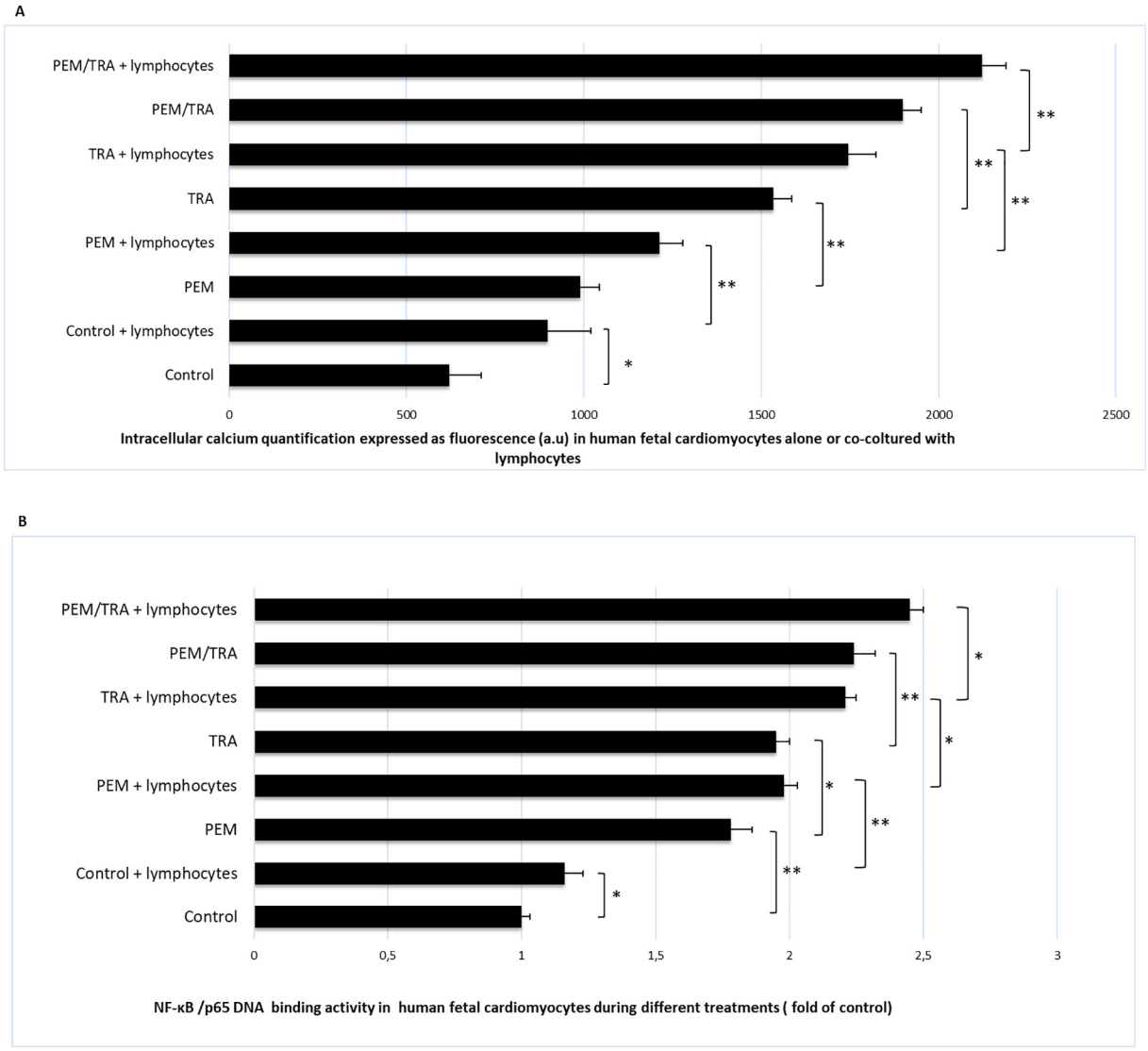

Fig. 2. A: Intracellular calcium staining by fluorescence methods in human fetal cardiomyocytes (expressed as fluorescence intensity a.u); B: effects of PEM, TRA or PEM/TRA on NF-Kb activation in cardiomyocytes. For both experiments, cells were co-cultured or not with lymphocytes and treated with or without PEM, TRA or PEM/TRA *$p < 0.05$, **$p < 0.001$; ns: not significant.

Specifically, in TRA-treated mice, IL-1β increases of 61.2% compared to untreated mice (Sham) ($p < 0.001$); in PEM group, heart IL-1β increases of 38.1% compared to Sham ($p < 0.001$) and combination therapy determined a more significant increase in IL-1β concentration of 138% compared to Sham ($p < 0.001$) and of 48% compared to TRA treatments.

A similar behavior was seen relatively to IL-6, with an increase of 47% in TRA-treated mice compared to Sham ($p < 0.001$) but without any statistical significant differences in PEM group (see Fig. 3). However, the combination therapy increased of two fold the IL-6 concentration, compared to Sham ($p < 0.001$), that was 1.3 fold greater compared to only TRA-treated mice ($p < 0.05$). Moreover, relatively to IL-8, we have seen a similar picture compared to the other interleukins, with an increase in its tissue production of 71.4% compared to untreated mice ($p < 0.001$); in PEM group, heart IL-8 was 36.7% more than untreated mice ($p < 0.001$). The association of PEM and TRA leads to an increase of 128.5% compared to untreated mice ($p < 0.001$) and to a difference of 33.2 and 67% compared to TRA and PEM treatments, respectively.

Leukotriene B4, a pro-inflammatory molecule having a key role in cardiovascular disease and cardiotoxicity [31], was quantified in cardiac lysates as shown in Fig. 3, E.

Treatment with TRA has doubled the heart tissue concentration of Leukotriene B4 compared to the untreated mice ($p < 0.001$) but without any significant effect in PEM-treated group (ns, not significant). However, the combination therapy PEM/TRA has additive pro-inflammatory effects increasing the Leukotriene B4 expression of 3 times compared to Sham ($p < 0.001$) and of 1.6 times compared to mice treated with TRA ($p < 0.001$). A similar behavior was seen relatively to the p65/NF-kB expression (Fig. 3, F) where treatments with TRA, PEM and their association have increased it of 2.6, 1.9 and 3.8 times compared to untreated mice, respectively ($p < 0.001$ for all). Also in this case, the combination of PEM and TRA significantly increased the inflammatory state compared to single treatments.

3.5. Cardiac fibrosis

Collagen deposition is a morphological marker of inflammation and is associated with diastolic and systolic dysfunction. The histological analysis showed an increased amount of collagen fibers in TRA and PEM/TRA groups (Fig. 4). While controls and PEM showed a typical intramyocardial collagen framework, with mainly type III collagen

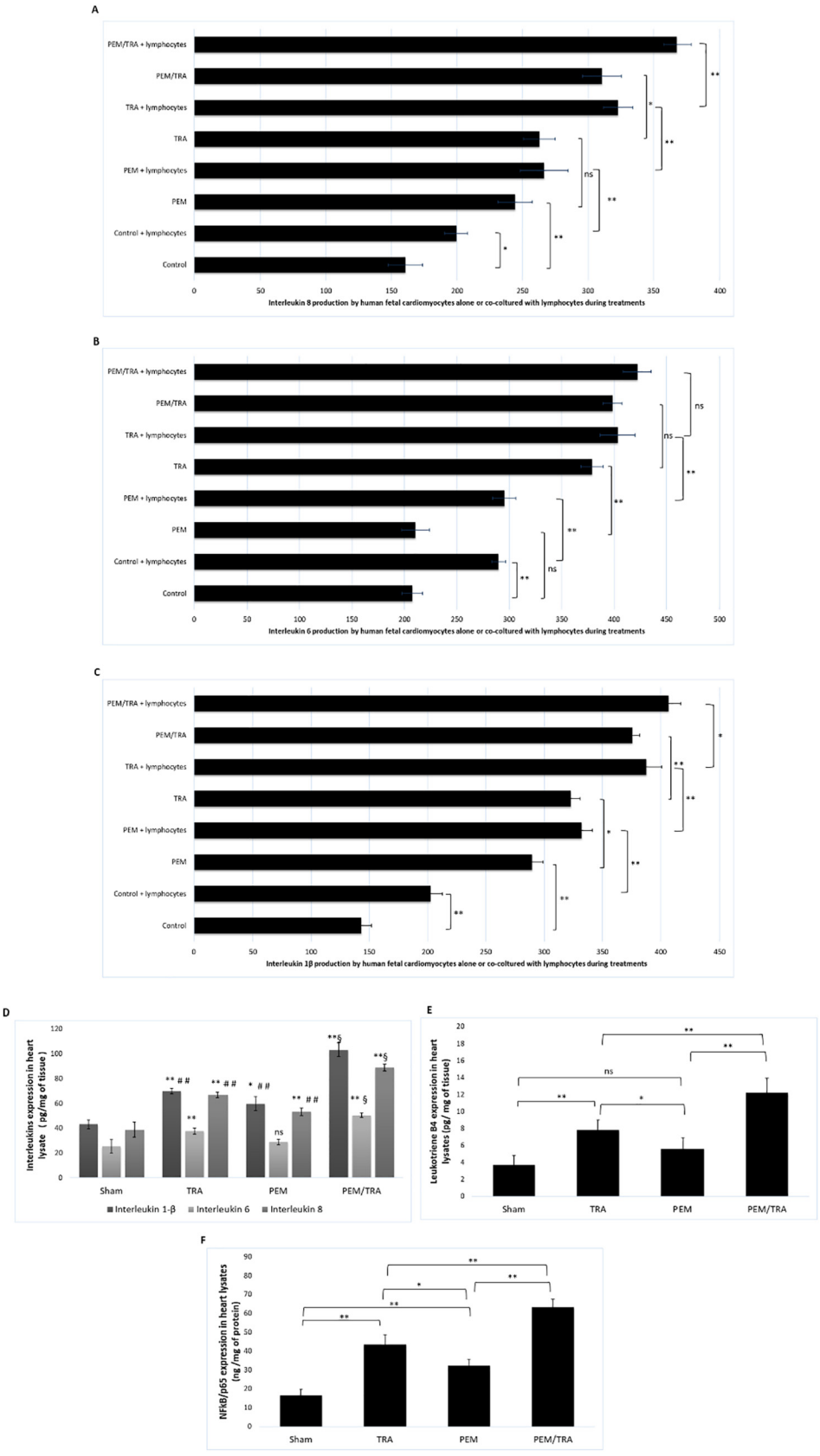

Fig. 3. Inflammation studies on human fetal cardiomyocytes co-cultured or not with lymphocytes and on cardiac tissues of mice treated with the mAbs. We quantified the IL-8 (A), IL-6 (B) and IL-1β (C) production by cardiomyocytes. Inflammation studies on the cardiac tissue extracts of mice untreated (Sham) or treated with TRA, PEM or PEM/TRA evaluating the production of interleukins (D) the Leukotriene B4 (E) and NFkB/p65 (F) expression. In Fig. A, B, C, E, and F *p < 0.05, **p < 0.001; ns: not significant; in Fig. D, *is the difference with the sham, #is the difference between PEM and TRA and §is the difference between PEM/TRA and TRA.

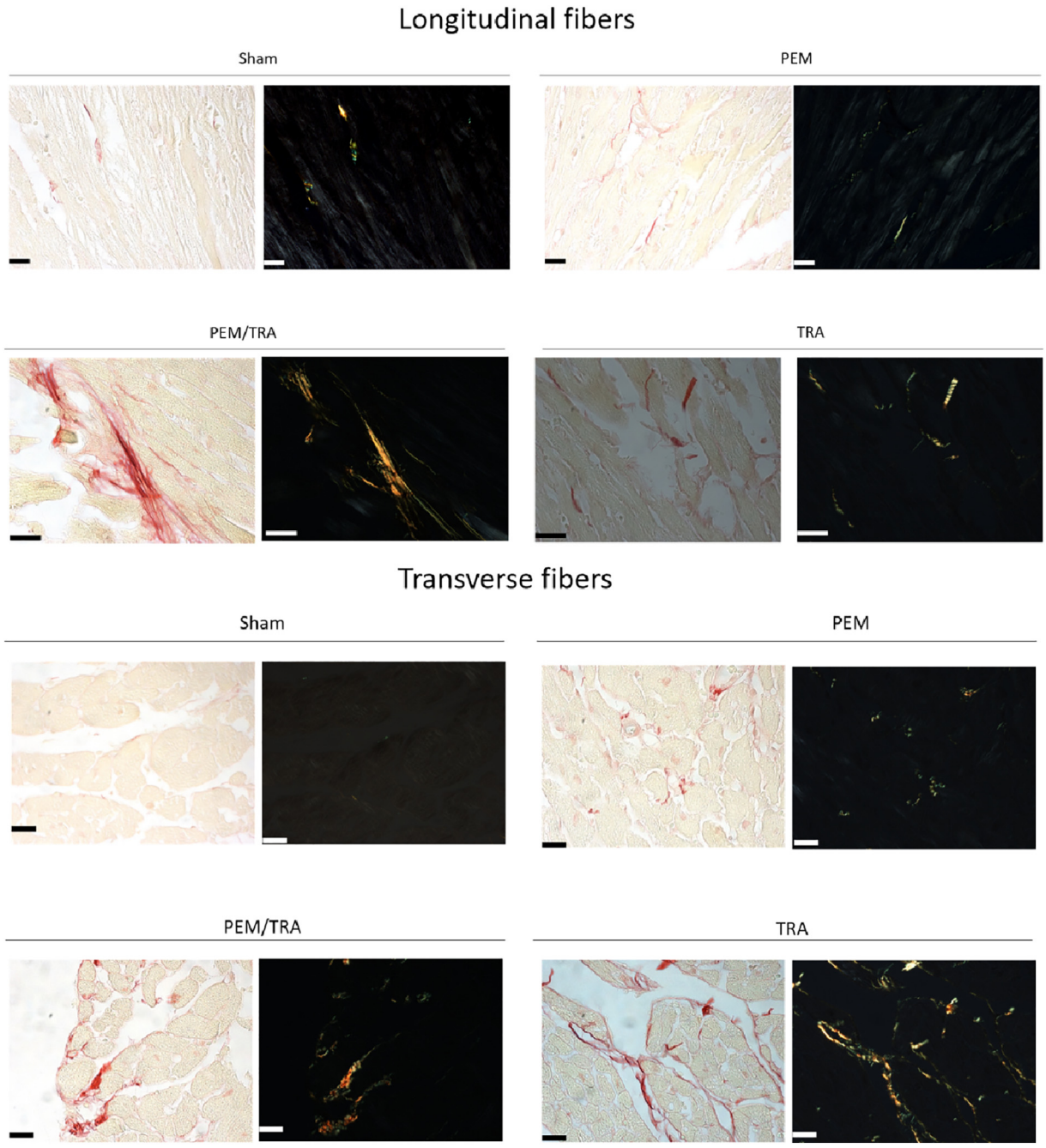

Fig. 4. Effects of PEM or PEM/TRA treatment on cardiac fibrosis. Collagen deposition in the perymisial space, stained with picrosirius red and observed under white (on the left) and polarized light (on the right). It is possible to distinguish between total collagen (pink), collagen I (red) and collagen III (green). Note the heavy collagen framework surrounding cells in TRA group, characterized by thick and dense fibers that is also present in the PEM/TRAS group Bar = 20 μm.

fibers in interstitial space, TRA and PEM/TRA groups showed a well-defined reactive fibrosis rich in Type I collagen fibers (Fig. 4).

4. Discussion

Immunotherapies are revolutionizing modern cancer treatments of several neoplasms, nevertheless the real incidence of early and late adverse events associated with immune checkpoint inhibitors are largely unknown. A recent review described the cardiac side effects of immunotherapies, with particular attention to the role of Tcells in the pathophysiology phenomena [32]. Non cardiac side effects of anti -PD-1 (pembrolizumab and nivolumab) and anti- PD-L1 drugs (atezolizumab) are reversible and mainly based on rash, pruritus, diarrhea and fatigue [33] and less frequently, on cases of endocrine-related diseases [34]. Other cases of autoimmune myocarditis were observed in patients treated with PEM and Ipilimumab [10]. Moreover, heart fibrosis, myocarditis or heart failure was recently seen in patients treated with anti-PD1 antibodies [35]. Combination therapy of anti PD-1 and anti CTLA-4 determined similar cases of fulminant myocarditis [36]; histological post mortem analysis demonstrated infiltration of lymphocytes in myocardium indicating a crucial role of the cardiomyocyte-lymphocyte interaction in the cardiotoxicity phenomena of anti-PD-1 or anti-CTLA-4 antibodies [36].

We demonstrated that cardiotoxic effects of the combinatorial treatment alters the intracellular calcium homeostasis in cardiomyocytes. Increases of intracellular calcium concentration ($[Ca^{2+}]i$) induces necrosis and apoptosis of cardiomyocytes by alteration of the mitochondrial redox state [37]. Notably, patients with myocarditis induced by

immunotherapies have been shown to have an expression of PD-1 on the myocyte membrane, confirming the involvement of PD-1/PDL-1 in the etiology of myocarditis [36]. Interestingly, the NF-κB activation in cardiomyocytes causes cardiomyopathy and heart failure by inducing an excessive inflammatory response and myocyte atrophy [38]. Pro-inflammatory chemokines, growth factors and interleukins, such as Interleukin 1-β, have a key role in the etiopathogenesis of myocarditis [39].

Treatment with antibodies against Interleukin 1-β (canakimumab) and Interleukin-6 (tocilizumab) leads to significant cardioprotection effects. Neutralization of IL-1 reduces acute and chronic myocarditis in mice [40]. Furthermore in "CANTOS-Trial", Canakinumab decreased the rate of recurrent cardiovascular events, compared to placebo [41]. Moreover, treatment with Tocilizumab demonstrated significant cardiovascular clinical benefits [42,43].

Preclinical and clinical studies have shown that leukotrienes are overexpressed during atherosclerosis, myocardial infarction and stroke [44]. Moreover, leukotrienes inhibitors showed significant cardioprotection in the preclinical trials [45], confirming the central role of leukotrienes in the pathophysiology of cardiac damage.

A recent clinical trial has shown that VIA-2291, a lipoxigenase inhibitor (the enzyme involved in the synthesis of Leukotrienes), reduced the plaque progression, compared with placebo, in patients with recent acute coronary syndrome, thus confirming the key role of Leukotrienes in atherosclerosis [46]. Notably, the receptor of Leukotriene B4 is involved in the invasiveness of breast cancer cells through IL-8 pathway [47].

Therefore the inhibition of a pro-inflammatory microenvironment, derived from Arachidonic acid and Leukotrienes-related pathways, both in cardiac and cancer tissues could be clinically beneficial in cancer patients. Interestingly, the CANTOS-Trial demonstrated, as secondary endpoint, a significant reduction of lung cancer incidence and mortality in patients treated with antibody against Interleukin-1 called Canakimumab [48].

Based on the data described herein, we can hypothesize that the novel regimen of combination therapy of anti-PD-1mAb and antibodies against tyrosine kinase receptors might exacerbate their cardiotoxic effects. Indeed, in addition to Trastuzumab also many other anti- tyrosine kinase drugs, such as Bevacizumab, Sunitinib, Sorafenib and Dasatinib, as recently described in literature [49], are characterized by well-known cardiotoxicity phenomena involving either Neuregulin-1 or inhibition of Adenosin monophosphate kinases-activated protein kinase which leads to apoptosis. However, preclinical or clinical data emphasizing this additive cardiotoxicity were not available in literature; experiments in this direction are being carried out by our research group.

A limitation of our work is based on the absence of studies of the standard cardiac parameters; in fact, quantification of plasma troponin and analysis of fractional shortening and ejection fraction by transthoracic echocardiography have been programmed by our research group in order to corroborate our preliminary cellular and biochemical data.

Considering the need for collaboration between cardiologists and oncologists on the cardiotoxicity of new targeted therapies [50], this study provides additional preliminary information to shed the light on the additive cardiotoxicity of different anticancer drugs.

The overall picture of this study turns the light on the cardiotoxic effects of immune check-point inhibitors especially when associated with Trastuzumab providing preliminary scientific evidences for the urgent need of cardiovascular monitoring strategies and cardiotoxicity management in cancer patients.

Funding statement

This work was funded by the "Ricerca Corrente" grant from the Italian Ministry of Health.

Declaration of Competing Interest

All the authors declare no conflict of interests.

References

[1] A. Ribas, J.D. Wolchok, Cancer immunotherapy using checkpoint blockade, Science. 359 (6382) (2018 Mar 23) 1350–1355, https://doi.org/10.1126/science.aar4060 (Epub 2018 Mar 22).
[2] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, W.J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma, N. Engl. J. Med. 363 (8) (2010 Aug 19) 711–723, https://doi.org/10.1056/NEJMoa1003466 (Epub 2010 Jun 5).
[3] D.B. Johnson, C. Peng, J.A. Sosman, Nivolumab in melanoma: latest evidence and clinical potential, Ther. Adv. Med. Oncol. 7 (2) (2015 Mar) 97–106, https://doi.org/10.1177/1758834014567469.
[4] C.L. Cowey, F.X. Liu, J. Black-Shinn, K. Stevinson, M. Boyd, J.R. Frytak, S.W. Ebbinghaus, Pembrolizumab utilization and outcomes for advanced melanoma in US community oncology practices, J. Immunother. 41 (2) (2018 Feb/Mar) 86–95, https://doi.org/10.1097/CJI.0000000000000204.
[5] J. Larkin, F.S. Hodi, J.D. Wolchok, Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, N. Engl. J. Med. 373 (13) (2015 Sep 24) 1270–1271, https://doi.org/10.1056/NEJMc1509660.
[6] M.A. Postow, R. Sidlow, M.D. Hellmann, Immune-related adverse events associated with immune checkpoint blockade, N. Engl. J. Med. 378 (2) (2018 Jan 11) 158–168, https://doi.org/10.1056/NEJMra1703481.
[7] S. Baxi, A. Yang, R.L. Gennarelli, N. Khan, Z. Wang, L. Boyce, D. Korenstein, Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: systematic review and meta-analysis, BMJ. 360 (2018 Mar 14) k793, https://doi.org/10.1136/bmj.k793.
[8] K. Haratani, H. Hayashi, Y. Chiba, K. Kudo, K. Yonesaka, R. Kato, H. Kaneda, Y. Hasegawa, K. Tanaka, M. Takeda, K. Nakagawa, Association of immune-related adverse events with nivolumab efficacy in non-small-cell lung cancer, JAMA Oncol. 4 (3) (2018 Mar 1) 374–378, https://doi.org/10.1001/jamaoncol.2017.2925.
[9] J.J. Moslehi, J.E. Salem, J.A. Sosman, B. Lebrun-Vignes, D.B. Johnson, Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis, Lancet. 391 (10124) (2018 Mar 10) 933, https://doi.org/10.1016/S0140-6736(18)30533-6.
[10] S.S. Mahmood, M.G. Fradley, J.V. Cohen, A. Nohria, K.L. Reynolds, L. Heinzerling, R.J. Sullivan, R. Damrongwatanasuk, C.L. Chen, D. Gupta, M.C. Kirchberger, M. Awadalla, Hassan MZO, J.J. Moslehi, S.P. Shah, S. Ganatra, P. Thavendiranathan, D.P. Lawrence, J.D. Groarke, T.G. Neilan, Myocarditis in patients treated with immune checkpoint inhibitors, J. Am. Coll. Cardiol. 71 (16) (2018 Apr 24) 1755–1764, https://doi.org/10.1016/j.jacc.2018.02.037. Epub 2018 Mar 19.
[11] C. Gravalos, A. Jimeno, HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target, Ann. Oncol. 19 (9) (2008 Sep) 1523–1529, https://doi.org/10.1093/annonc/mdn169 (Epub 2008 Apr 25).
[12] G. Riccio, A.R. Da Fonseca-Ricardo, M. Passariello, P. Cunnah, N. Mertens, C. De Lorenzo, Superior suppression of ErbB2-positive tumor cells by a novel human triparatopic tribody, J. Immunother. (2017 Jan 25)https://doi.org/10.1097/CJI.0000000000000152.
[13] G. Riccio, G. Esposito, E. Leoncini, R. Contu, G. Condorelli, M. Chiariello, P. Laccetti, S. Hrelia, G. D'Alessio, C. De Lorenzo, Cardiotoxic effects, or lack thereof, of anti-ErbB2 immunoagents, FASEB J. 23 (9) (2009 Sep) 3171–3178, https://doi.org/10.1096/fj.09-131383 (Epub 2009 May 5).
[14] C. Fedele, G. Riccio, A.E. Malara, G. D'Alessio, C. De Lorenzo, Mechanisms of cardiotoxicity associated with ErbB2 inhibitors, Breast Cancer Res. Treat. 134 (2) (2012 Jul) 595–602, https://doi.org/10.1007/s10549-012-2103-8 (Epub 2012 Ju).
[15] C. De Lorenzo, R. Paciello, G. Riccio, D. Rea, A. Barbieri, C. Coppola, N. Maurea, Cardiotoxic effects of the novel approved anti-ErbB2 agents and reverse cardioprotective effects of ranolazine, Onco. Targets Ther. 11 (2018 Apr 19) 2241–2250, https://doi.org/10.2147/OTT.S157294 (eCollection 2018).
[16] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, L. Norton, Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (11) (2001 Mar 15) 783–792.
[17] Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro G Jr, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, McFadden E, Leyland-Jones B, Bell R, Dowsett M, Jackisch C, Herceptin Adjuvant (HERA) Trial Study Team. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin adjuvant (HERA) trial. Lancet. 2017 Mar 25; 389(10075):1195–1205.
[18] G. Riccio, C. Coppola, G. Piscopo, I. Capasso, C. Maurea, E. Esposito, C. De Lorenzo, N. Maurea, Trastuzumab and target-therapy side effects: is still valid to differentiate anthracycline type I from type II cardiomyopathies? Hum. Vaccin. Immunother. 12 (5) (2016 May 3) 1124–1131, https://doi.org/10.1080/21645515.2015.1125056 (Epub 2016 Feb 2).
[19] C.G. Tocchetti, G. Ragone, C. Coppola, D. Rea, G. Piscopo, S. Scala, C. De Lorenzo, R.V. Iaffaioli, C. Arra, N. Maurea, Detection, monitoring, and management of trastuzumab-induced left ventricular dysfunction: an actual challenge, Eur. J. Heart Fail. 14 (2) (2012 Feb) 130–137, https://doi.org/10.1093/eurjhf/hfr165 (Epub 2012 Jan 4).
[20] Tocchetti CG, Coppola C, Arra C, Maurea N. Early identification of left ventricular dysfunction induced by trastuzumab. J. Am. Coll. Cardiol. 2011 Dec 13;58(25):

2698–9; author reply 2699–700. doi: https://doi.org/10.1016/j.jacc.2011.08.056. No abstract available.
[21] S Loi, A Giobbe-Hurder, A Gombos, T Bachelot, R Hui, G Curigliano, M Campone, L Biganzoli, H Bonnefoi, G Jerusalem, R Bartsch, M Rabaglio-Poretti, R Kammler, R Maibach, MJ Smyth, A Di Leo, M Colleoni, G Viale, MM Regan and F Andre. Phase Ib/II study evaluating safety and efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant HER2-positive metastatic breast cancer: Results from the PANACEA (IBCSG 45-13/BIG 4-13/KEYNOTE-014) study. Cancer Research, February 2018 Volume 78, Issue 4 Supplement. DOI: https://doi.org/10.1158/1538-7445.SABCS17-GS2-06 Published February 2018.
[22] Quagliariello V, Vecchione R, Coppola C, Di Cicco C, De Capua A, Piscopo G, Paciello R, Narciso V, Formisano C, Taglialatela-Scafati O, Iaffaioli RV, Botti G, Netti PA, Maurea N. Cardioprotective effects of nanoemulsions loaded with anti-inflammatory nutraceuticals against doxorubicin-Induced cardiotoxicity nutrients. 2018 Sep 14; 10(9). pii: E1304. doi: https://doi.org/10.3390/nu10091304.
[23] V. Quagliariello, R.V. Iaffaioli, E. Armenia, O. Clemente, M. Barbarisi, G. Nasti, M. Berretta, A. Ottaiano, A. Barbarisi, Hyaluronic acid nanohydrogel loaded with quercetin alone or in combination to a macrolide derivative of rapamycin RAD001 (Everolimus) as a new treatment for hormone-responsive human breast Cancer, J. Cell. Physiol. 232 (8) (2017 Aug) 2063–2074, https://doi.org/10.1002/jcp.25587 (Epub 2017 Mar 1).
[24] D. Rea, C. Coppola, A. Barbieri, M.G. Monti, G. Misso, G. Palma, S. Bimonte, M.R. Zarone, A. Luciano, D. Liccardo, P. Maiolino, A. Cittadini, G. Ciliberto, C. Arra, N. Maurea, In Vivo 30 (3) (2016 May-Jun) 279–290.
[25] Coppola C, Riccio G, Barbieri A, Monti MG, Piscopo G, Rea D, Arra C, Maurea C, De Lorenzo C, Maurea N. Antineoplastic-related cardiotoxicity, morpho functional aspects in a murine model: contribution of the new tool 2D-speckle tracking. Onco. Targets Ther. 2016 Nov 2;9:6785–6794. (eCollection 2016).
[26] M. Wang, L.C. Yao, M. Cheng, D. Cai, J. Martinek, C.X. Pan, W. Shi, A.H. Ma, R.W. De Vere White, S. Airhart, E.T. Liu, J. Banchereau, M.A. Brehm, D.L. Greiner, L.D. Shultz, K. Palucka, J.G. Keck, Humanized mice in studying efficacy and mechanisms of PD-1-targeted cancer immunotherapy, FASEB J. 32 (3) (2018 Mar) 1537–1549, https://doi.org/10.1096/fj.201700740R (Epub 2018 Jan 3).
[27] A.K. Syed, R. Woodall, J.G. Whisenant, T.E. Yankeelov, A.G. Sorace, Characterizing trastuzumab-induced alterations in intratumoral heterogeneity with quantitative imaging and immunohistochemistry in HER2+ breast cancer, Neoplasia. 21 (1) (2019 Jan) 17–29.
[28] Seo JM, Park S, Kim JH. Leukotriene B4 receptor-2 promotes invasiveness and metastasis of ovarian cancer cells through signal transducer and activator of transcription 3 (STAT3)-dependent up-regulation of matrix metalloproteinase 2. J. Biol. Chem. 2012 Apr 20;287(17):13840–9. doi: https://doi.org/10.1074/jbc.M111.317131. Epub 2012 Mar 6.
[29] de Melo Gagliato D, Cortes J, Curigliano G, Loi S, Denkert C, Perez-Garcia J, Holgado E. Tumor-infiltrating lymphocytes in breast cancer and implications for clinical practice. Biochim. Biophys. Acta, Rev. Cancer 2017 Dec;1868(2):527–537. doi: https://doi.org/10.1016/j.bbcan.2017.10.003. (Epub 2017 Oct 20).
[30] M.J. Selby, J.J. Engelhardt, R. Johnston, L.S. Lu, M. Han, K. Thudium, D. Yao, M. Quigley, J. Valle, C. Wang, B. Chen, P.M. Cardarelli, D. Blanset, A.J. Korman, Preclinical development of ipilimumab and nivolumab combination immunotherapy: mouse tumor models, in vitro functional studies, and cynomolgus macaque toxicology, PLoS One 11 (9) (2016 Sep 9), e0161779. https://doi.org/10.1371/journal.pone.0161779 (eCollection 2016).
[31] E. Dvash, M. Har-Tal, S. Barak, O. Meir, M. Rubinstein, Leukotriene C4 is the major trigger of stress-induced oxidative DNA damage, Nat. Commun. 6 (2015 Dec 11), 10112. https://doi.org/10.1038/ncomms10112.
[32] K.H. Simons, A. de Jong, J.W. Jukema, M.R. de Vries, R. Arens, P.H.A. Quax, T cell co-stimulation and co-inhibition in cardiovascular disease: a double-edged sword, Nat. Rev. Cardiol. (2019 Feb 15) https://doi.org/10.1038/s41569-019-0164-7. Review.
[33] M.E. Lacouture, J.D. Wolchok, G. Yosipovitch, K.C. Kähler, K.J. Busam, A. Hauschild, Ipilimumab in patients with cancer and the management of dermatologic adverse events, J. Am. Acad. Dermatol. 71 (1) (2014) 161–169.
[34] J.S. Weber, K.C. Kähler, A. Hauschild, Management of immune-related adverse events and kinetics of response with ipilimumab, J. Clin. Oncol. 30 (21) (2012) 2691–2697.
[35] C.J. Voskens, S.M. Goldinger, C. Loquai, C. Robert, K.C. Kaehler, C. Berking, T. Bergmann, C.L. Bockmeyer, T. Eigentler, M. Fluck, C. Garbe, R. Gutzmer, S. Grabbe, A. Hauschild, R. Hein, G. Hundorfean, A. Justich, U. Keller, C. Klein, C. Mateus, P. Mohr, S. Paetzold, I. Satzger, D. Schadendorf, M. Schlaeppi, G. Schuler, B. Schuler-Thurner, U. Trefzer, J. Ulrich, J. Vaubel, R. von Moos, P. Weder, T. Wilhelm, D. Göppner, R. Dummer, L.M. Heinzerling, The price of tumor control: an analysis of rare side effects of anti CTLA-4 therapy in metastatic melanoma from the ipilimumab network, PLoS One 8 (1) (2013), e53745.
[36] D.Y. Wang, J.E. Salem, J.V. Cohen, S. Chandra, C. Menzer, F. Ye, S. Zhao, S. Das, K.E. Beckermann, L. Ha, W.K. Rathmell, K.K. Ancell, J.M. Balko, C. Bowman, E.J. Davis, D.D. Chism, L. Horn, G.V. Long, M.S. Carlino, B. Lebrun-Vignes, Z. Eroglu, J.C. Hassel, A.M. Menzies, J.A. Sosman, R.J. Sullivan, J.J. Moslehi, D.B. Johnson, Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis, JAMA Oncol. 4 (12) (2018 Dec 1) 1721–1728, https://doi.org/10.1001/jamaoncol.2018.3923.
[37] A. Maillet, K. Tan, X. Chai, S.N. Sadananda, A. Mehta, J. Ooi, M.R. Hayden, M.A. Pouladi, S. Ghosh, W. Shim, L.R. Brunham, Modeling doxorubicin-induced cardiotoxicity in human pluripotent stem cell derived-cardiomyocytes, Sci. Rep. 6 (2016 May 4), 25333. https://doi.org/10.1038/srep25333.
[38] H.J. Maier, T.G. Schips, A. Wietelmann, M. Krüger, C. Brunner, M. Sauter, K. Klingel, T. Böttger, T. Braun, T. Wirth, Cardiomyocyte-specific IκB kinase (IKK)/NF-κB activation induces reversible inflammatory cardiomyopathy and heart failure, Proc. Natl. Acad. Sci. U. S. A. 109 (29) (2012 Jul 17) 11794–11799, https://doi.org/10.1073/pnas.1116584109.
[39] G. De Luca, G. Cavalli, C. Campochiaro, M. Tresoldi, Dagna L. Myocarditis, An interleukin-1-mediated disease? Front. Immunol. 9 (2018 Jun 13) 1335, https://doi.org/10.3389/fimmu.2018.01335.
[40] L. Kraft, T. Erdenesukh, M. Sauter, C. Tschöpe, K. Klingel, Blocking the IL-1β signalling pathway prevents chronic viral myocarditis and cardiac remodeling, Basic Res. Cardiol. 114 (2) (2019 Jan 23) 11, https://doi.org/10.1007/s00395-019-0719-0.
[41] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, F. Fonseca, J. Nicolau, W. Koenig, S.D. Anker, J.J.P. Kastelein, J.H. Cornel, P. Pais, D. Pella, J. Genest, R. Cifkova, A. Lorenzatti, T. Forster, Z. Kobalava, L. Vida-Simiti, M. Flather, H. Shimokawa, H. Ogawa, M. Dellborg, P.R.F. Rossi, R.P.T. Troquay, P. Libby, R.J. Glynn, CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease, N. Engl. J. Med. 377 (12) (2017 Sep 21) 1119–1131, https://doi.org/10.1056/NEJMoa1707914 (Epub 2017 Aug 27).
[42] A. Fioravanti, S. Tenti, M.R. Bacarelli, A. Damiani, F. Li Gobbi, F. Bandinelli, S. Cheleschi, M. Galeazzi, M. Benucci, Tocilizumab modulates serum levels of adiponectin and chemerin in patients with rheumatoid arthritis: potential cardiovascular protective role of IL-6 inhibition, Clin. Exp. Rheumatol. 37 (2) (2019 Mar-Apr) 293–300.
[43] B.C. Bacchiega, A.B. Bacchiega, M.J. Usnayo, R. Bedirian, G. Singh, G.D. Pinheiro, Interleukin 6 inhibition and coronary artery disease in a high-risk population: a prospective community-based clinical study, J. Am. Heart Assoc. 6 (3) (2017 Mar 13) https://doi.org/10.1161/JAHA.116.005038 pii: e005038.
[44] H. Hakonarson, S. Thorvaldsson, A. Helgadottir, Effects of a 5-lipoxygenase–activating protein inhibitor on biomarkers associated with risk of myocardial infarction: a randomized trial, JAMA 293 (18) (2005) 2245–2256.
[45] M. Hoxha, G.E. Rovati, A.B. Cavanillas, The leukotriene receptor antagonist montelukast and its possible role in the cardiovascular field, Eur. J. Clin. Pharmacol. 73 (7) (2017) 799–809.
[46] S. Matsumoto, R. Ibrahim, J.C. Grégoire, P.L. L'Allier, J. Pressacco, J.C. Tardif, M.J. Budoff, Effect of treatment with 5-lipoxygenase inhibitor VIA-2291 (atreleuton) on coronary plaque progression: a serial CT angiography study, Clin. Cardiol. 40 (4) (2017 Apr) 210–215, https://doi.org/10.1002/clc.22646 (Epub 2016 Nov 24).
[47] J. Park, J.H. Jang, G.S. Park, Y. Chung, H.J. You, J.H. Kim, BLT2, a leukotriene B4 receptor 2, as a novel prognostic biomarker of triple-negative breast cancer, BMB Rep. 51 (8) (2018 Aug) 373–377.
[48] P.M. Ridker, J.G. MacFadyen, T. Thuren, B.M. Everett, P. Libby, Glynn RJ; CANTOS Trial Group. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial, Lancet. 390 (10105) (2017 Oct 21) 1833–1842, https://doi.org/10.1016/S0140-6736(17)32247-X.
[49] N. Maurea, C. Coppola, G. Piscopo, F. Galletta, G. Riccio, E. Esposito, C. De Lorenzo, M. De Laurentiis, P. Spallarossa, G. Mercuro, Pathophysiology of cardiotoxicity from target therapy and angiogenesis inhibitors, J. Cardiovasc. Med. (Hagerstown) 17 (Suppl. 1) (2016 May) e19–e26 Special issue on Cardiotoxicity from Antiblastic Drugs and Cardioprotection.
[50] N. Maurea, C. Coppola, G. Ragone, G. Frasci, A. Bonelli, C. Romano, R.V. Iaffaioli, Women survive breast cancer but fall victim to heart failure: the shadows and lights of targeted therapy, J. Cardiovasc. Med. (Hagerstown) 11 (12) (2010 Dec) 861–868, https://doi.org/10.2459/JCM.0b013e328336b4c1.