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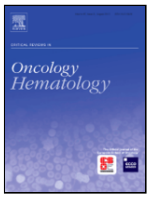

Lymphopenia and intratumoral lymphocytic balance in the era of cancer immuno-radiotherapy

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ABSTRACT

Introduction: The immune response has been recognized as a major tumor-eradication component of radiotherapy.
Objective: This review studies, under a clinical perspective, two contrasting effects of radiotherapy, namely immunosuppression and radiovaccination.
Materials and methods: We critically reviewed the available clinical and experimental experience on radiotherapy-induced lymphopenia.
Results: Radiation-induced tumor damage promotes radio-vaccination, enhances cytotoxic immune responses, and potentiates immunotherapy. Nevertheless, radiotherapy induces systemic and intratumoral lymphopenia. The above effects are directly related to radiotherapy fractionation and field size/location, and tumor characteristics.
Discussion: Hypofractionated stereotactic and accelerated irradiation better promotes radio-vaccination and produces less severe lymphopenia. Adopting cytoprotective policies and combining lympho-stimulatory agents or agents blocking regulatory lymphocyte activity are awaited to unmask the radio-vaccination effect, enhancing the efficacy immuno-radiotherapy.
Conclusion: Radiation-induced lymphopenia and immunosuppression are important issues that should be considered in the design of immuno-radiotherapy clinical trials.

1. Introduction

Experimental mice's natural resistance to receive syngeneic tumor transplants is well documented since the beginning of the 20th century (Bashford et al., 1907). This phenomenon was directly related to the number of injected tumor cells and host characteristics. Age, site of transplantation, or previous exposure to tumor cells defined the robustness of this 'natural resistance.' It took more than half a century for researchers to recognize lymphoblast-like cells that could attack and destroy cancer cells *in vitro* (Berczi et al., 1973). White blood cells were revealed as the tissue responsible for tumor rejection (Schirrmacher et al., 1981). Specific phenotypes of cells of lymphocytic and monocytic origin co-operate in a network of interactions of extreme complexity to define cancer tolerance or rejection. Both the innate and adaptive immune pathways are involved in tumor rejection. The innate immune response is mediated by non-specific, antigen-independent, attack by Natural Killer NK-cells (CD56+ lymphocytes) and macrophages (Martinet and Smyth, 2015). The adaptive immune response is mediated by activated T-cells (mainly CD8+, but also CD4 lymphocytes) that recognize foreign antigens on cancer cell membranes. The immune system also develops a specific subtype of T-cells, the so-called regulatory T-cells (Tregs) expressing mainly CD4, FOXP3 and CD25 antigens (Chen, 2011), that suppress the activity of cytotoxic T-cells.

Although an intact and properly functioning lymphocytic immune system is important for immune surveillance, cancer cells may evade cytotoxic cells by undergoing phenotypic changes, the so-called immunoediting (O'Donnell et al., 2019). Loss of recognition molecules by cancer cells, like loss of HLA-class-I molecules (Kaklamanis et al., 1995), or overexpression of immune checkpoint inhibitory molecules, like PD-L1 and CD80, are important pathways exploited by cancer cells to escape immune surveillance (Dyck and Mills, 2017). This later type of molecule has become the main target for modern immunotherapy with immune checkpoint inhibitors (ICIs). Nevertheless, the amount of cancer-targeting lymphocytes that reach the tumor remains an important parameter defining the immunotherapy efficacy. Saturation of the immune system by large cancer cell loads and rapid growth of tumors is one of the principal causes of immunotherapy failure (Costello et al., 1999). Such saturation occurs more readily in lym-

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phopenic patients. Lymphopenia is an important condition that should be recognized and corrected to assist tumor rejection of patients undergoing radiotherapy, chemotherapy, and immunotherapy.

2. Objectives

Here we review clinical and experimental studies that have focused on radiotherapy-related parameters producing two contrasting radiotherapy effects, namely lymphopenia and radio-vaccination. Moreover, we summarize different approaches that could be developed clinically to minimize radiation-induced lymphopenia and reverse immunosuppression and regulatory immune cell responses. Overall, the background of radiation interactions with tumor microenvironment and host immunity is critically reviewed to provide a basis to take into account for the design of clinical immuno-radiotherapy trials.

3. Materials and methods

The literature search was performed in the EMBASE and MEDLINE databases using the text-words "radiotherapy", "hypofractionation", "SBRT", "proton", "chemotherapy", "immune response", "immunotherapy", "cytoprotection","tumor microenvironment", "lymphopenia", and "lymphocytes".

4. Results

4.1. Pre-treatment lymphopenia

In the early 60's Stansly PG published an article focusing on the immunological tolerance of host during cancer development (Stansly, 1963). Several reports in the 70's brought forward the eventual role of tumor-induced immunosuppression in cancer patients and the necessity to identify the causes and restore immunocompetency (Simmler and Bruley-Rosset, 1976; Kerbel, 1974). Although tumor-induced immunosuppression is founded on the impaired balance between regulatory lymphocytes or abnormal/immature myeloid cells and cytotoxic cells (discussed later in this review), lymphopenia is also a component of the phenomenon. In 1972, Brooks et al. reported that a heat-stable factor in the sera of half of the patients with intracranial tumors blocks the blastogenic effect of phytohemagglutinin (Brooks et al., 1972). Of interest, when lymphocytes were washed and placed in normal plasma, their responsiveness was restored. Bambury et al. analyzed a series of 137 glioblastoma patients showing that lower pre-corticosteroid lymphocyte counts, as assessed by the neutrophil to lymphocyte ratio, were linked with poor prognosis (Bambury et al., 2013). Pediatric medulloblastoma patients have significantly lower lymphocyte counts compared to controls (Patel et al., 2018). In an interesting study by Joseph et al., pre-treatment lymphopenia in patients with muscle-invasive bladder cancer treated with radical chemo-radiotherapy or patients with advanced disease treated with palliative chemotherapy was an independent factor or poor prognosis in both situations (Joseph et al., 2016). An analysis of 260 patients with colorectal cancer showed that 19 % of patients suffer from pre-treatment lymphopenia. This feature was directly linked with treatment-related hematological toxicity and shorter progression-free interval (Cézé et al., 2011). Holub et al. reported on a series of 155 patients with endometrial cancer treated with postoperative radiotherapy, where lymphopenia was associated with an unfavorable prognosis (Holub et al., 2020). In an analysis of 153 patients with treatment-naïve head-neck cancer undergoing chemo-radiotherapy, pre-treatment high neutrophil/lymphocyte ratio was an independent factor of poor progression-free survival (Moon et al., 2016).

Further to the tumor-induced lymphopenia, cancer patients may also be lymphopenic due to the immunosuppressive medication received for other benign diseases and conditions. For example, patients with inflammatory bowel disease or rheumatic diseases are linked with a high incidence of cancer and are often under chronic medication with immunosuppressive agents. Anti-TNF-α administration induces apoptosis of lymphocytes (Vigna-Pérez et al., 2005). Cyclophosphamide and azathioprine used in connective tissue diseases also induce lymphopenia (Hurd and Giuliano, 1975; Vögelin et al., 2016).

4.2. Treatment-induced lymphopenia

Radiotherapy and chemotherapy are the leading causes of lymphopenia in cancer patients. Induction of lymphopenia in patients receiving cranial radiotherapy or radiotherapy for head-neck cancer has been recognized since the '70 s (Harisiadis et al., 1977; Tarpley et al., 1975). Since then, many studies have focused on this ominous side effect of radiotherapy [reviewed in 25]. Lymphocytes are quite sensitive to radiation as, despite the variation existing between individuals, a fraction of 2 Gy kills 50 % of the irradiated population (Pouliliou et al., 2015). Radiotherapy-induced lymphopenia is a direct consequence of the irradiation of the blood passing through the irradiated body area during radiotherapy. Indeed, extracorporeal irradiation of blood routinely performed for the blood transfusion of immunosuppressed patients, eliminates lymphocytes and graft-versus-host disease (Asai et al., 2000). The exposure of the bone marrow or spleen comprised within, or located proximal to the radiotherapy fields is also important. Low dose radiation dispersed outside the radiation portals (around 5–10 % of the tumor dose) also affects lymphocytes. Splenic irradiation during radiotherapy for hepatocellular, pancreatic, or gastric cancer is linked with protracted lymphocytopenia, so the spleen should be considered as an organ at risk in the radiotherapy planning (Liu et al., 2017; Chadha et al., 2017). Aside from the direct killing effect of radiation on lymphocytes, immunosuppressive proteins, like Galectin-1, secreted by the irradiated cancer cells, have been also postulated to contribute to T-cell apoptosis and lymphopenia (Kuo et al., 2014).

Most chemotherapeutic drugs induce lymphopenia. Progressive lymphopenia in breast cancer patients was noted during treatment with the traditional CMF regimen (Blomgren et al., 1988). Intense lymphopenia is observed in patients treated with a combination of cisplatin with bleomycin or taxanes (Najarian et al., 1981). Docetaxel monotherapy induces profound but reversible lymphopenia that concerns all types of lymphocytes (Kotsakis et al., 2000). Lymphopenia is also a worrying side effect of taxane combination with radiotherapy (Koukourakis et al., 1999). Half of the patients receiving antimetabolite chemotherapy with pemetrexed and gemcitabine develop grade 3–4 lymphopenia (Hensley et al., 2008). Capecitabine also induces lymphopenia (Koizumi et al., 2003), which is also a common adverse effect of FOLFOX chemotherapy for colorectal cancer (Garcia-Aguilar et al., 2015). Novel targeted agent administration may also induce lymphotoxicity. Anti-EGFR therapy produces lymphopenia in 30 % of patients (Ho et al., 2011). Half of patients treated with anti-VEGF therapy develop lymphopenia (Reismüller et al., 2010). Multi-tyrosin kinase inhibitors can also produce grade 3–4 lymphopenia in 10–15 % of patients (Guevremont et al., 2009). mTOR inhibitors lead to lymphopenia in less than 10 % of patients (Guo et al., 2013). As combinations of immunotherapy with chemotherapy gradually become a common therapeutic practice, chemotherapy-induced lymphopenia should focus our thorough attention.

Immunotherapy per se may affect the quantity and quality of circulating lymphocytes. Administration of IFNα seems to produce mild leukopenia, but there is no clear data about its effect on lymphocyte counts. On the contrary, another important cytokine, IL2, approved for renal cancer treatment, seems to induce a biphasic response with an initial phase of lymphopenia, followed by an increase of lymphocyte counts (Sano et al., 1988). Despite the expected stimulatory effect of the above cytokines on lymphocytes, several data show that IL-2 may increase regulatory T-cells (Westermann et al., 2001), which brings forward important questions on the schedules and doses applied to avoid immunosuppression during immunotherapy. The design of al-

tered IL-2 molecules that do not bind on IL-2Ra receptor on regulatory T-cells may be important to shift IL-2 activity towards effector lymphocytic response (Setti et al., 1999). Combined administration of IFNα, IL2, and GM-CSF results in increased counts of CD3+, CD4+, CD8+, and CD56+ leukocytes following several weeks of therapy (Ahmadzadeh and Rosenberg, 2006). GM-CSF is an important cytokine that, further to its potent stimulatory activity on granulocytes and monocytes, restores the lymphocytes' proliferative capacity (Heaton et al., 1993a).

4.3. Lymphopenia and response to radiotherapy and chemotherapy

In the '70 s, experimental studies reported that the dose of radiotherapy demanded to eradicate a tumor on immunocompromised mice was twice as high as the one demanded in immunocompetent mice (Suit and Kastelan, 1970; Stone et al., 1979; Suit et al., 1990). Immunogenic death, aside to direct cell killing, has been recognized as a major component of tumor rejection after cytotoxic therapy. Clinical studies have also shown a strong reduction in the efficacy of radiotherapy in immunosuppressed patients (Tseng et al., 2018; Manyam et al., 2017; Ramey et al., 2015).

Recently published review articles summarize the adverse effect of lymphopenia on the results of radiotherapy and chemotherapy for solid tumors (Venkatesulu et al., 2018; Ménétrier-Caux et al., 2019). Among such studies, Liu et al. reported a 2.9 fold increased risk for tumor progression after radiotherapy in a series of 413 nasopharyngeal cancer patients when they suffered from grade 3/4 lymphopenia (Liu et al., 2018). In a series of 711 patients with NSCLC treated with radiotherapy, lymphopenia was related to worse overall survival (Tang et al., 2014). Baseline lymphopenia was an indicator of poor survival in a series of 122 patients with small-cell lung cancer treated with chemotherapy and radiotherapy (Suzuki et al., 2019). However, Ng et al. did not record any association of lymphopenia with survival following radiotherapy for oropharyngeal cancer (Ng et al., 2020).

Lymphopenia developed after induction chemotherapy was linked with incomplete surgical tumor removal in patients with ovarian cancer (Yoshino et al., 2019). Low lymphocyte counts were among the parameters defining treatment failure and poor overall survival in patients with Hodgkin's lymphoma treated with ABVD chemotherapy (Bhethanabhotla et al., 2017). Patients with baseline lymphopenia treated with chemotherapy for metastatic esophageal cancer have a poorer survival (Kou et al., 2016). Triple-negative breast cancer patients with low lymphocyte counts also seem to have a shorter survival (Patel et al., 2019). Baseline lymphopenia is a feature related to poor outcomes in breast cancer patients treated with trastuzumab for metastatic disease (Che et al., 2019). Ray-Coquard et al. reported three randomized trials performed in France, showing that lymphopenia defined poor prognosis in patients with advanced carcinomas, sarcomas, and lymphomas (Ray-Coquard et al., 2009).

4.4. Effect of lymphopenia on the efficacy of immunotherapy

Anti-PD1 and anti-PD-L1 immune checkpoint inhibitors are widely applied for the treatment of solid tumors. Unfortunately, the largest phase 2 and 3 trials with such agents do not report on lymphocyte counts and their predictive role. Even phase 3 studies comparing immunotherapies vs. the heavily lymphotoxic taxanes, do not comment on an eventual sparing effect of ICIs on lymphocytes (Borghaei et al., 2015). Our experience with anti-PD-1 immunotherapy shows that lymphopenia occurs in a small percentage of cases within the first year of its administration. This is an important difference between ICI immunotherapy and chemotherapy, as the latter strongly suppress lymphocytes and, eventually, promotes cancer immune escape in subgroups of patients. High doses of ICIs, like 10–20 mg/kg of nivolumab seem, however, to result in lymphopenia in 59 % of patients (Yamamoto et al., 2017).

Nevertheless, the role of lymphopenia in the response of tumors to ICIs has been examined in smaller studies. In a study by Ho et al. on 34 patients with head-neck cancer treated with ICIs, pre-treatment low lymphocyte counts were linked with inferior benefit, mostly when counts were $<600/\mu l$ (Ho et al., 2018). The progression-free interval was half to the one recorded in patients with higher lymphocyte counts in this latter category. Karantanos et al. reported a study on 22 patients with advanced NSCLC treated with nivolumab (Karantanos et al., 2019). At baseline or six weeks after the onset of immunotherapy, low lymphocyte counts were linked with poorer overall survival. Diehl et al. reported the results of ICI-therapy on 167 adults with solid tumors (Diehl et al., 2017). Patients with lymphopenia at baseline or persistent lymphopenia during immunotherapy had a shorter time to disease progression. In a study by Sun et al. on 167 patients treated with ICIs, a trend for poorer response rates and survival was noted for patients with baseline lymphopenia, but this did not reach statistical significance (Sun et al., 2017).

Of interest, high LDH plasma levels link with the low efficacy of ICIs in several studies (Bigot et al., 2017; Weide et al., 2016). These data show that, further to systemic lymphocytopenia, anaerobic tumor metabolism and its partner 'intratumoral lymphopenia' is the sister face of the same enemy of immunotherapy. High tumor anaerobic metabolism, as assessed by high LDHA, glycolysis enzyme, and monocarboxylate transporter expression in non-small cell lung cancer, has been linked with low tumor-infiltrating lymphocyte density (Giatromanolaki et al., 2019).

4.5. Lymphopenia and immuno-radiotherapy

Cho et al. published a study on 268 patients with advanced NSCLC treated with ICI-immunotherapy, where 146 of them also received radiotherapy (Cho et al., 2019). Patients with lymphopenia at the beginning of immunotherapy had a significantly poorer progression-free survival (2.2 vs. 5.9 months). When applied to multiple sites and given in multiple courses, radiotherapy was the principal cause of lymphopenia. Similar results have been reported by Pike et al., who found that prolonged radiotherapy courses before immunotherapy increase the risk of severe lymphopenia that is significantly related to poorer survival of patients receiving ICIs (Pike et al., 2019). Radiotherapy before immunotherapy was also linked with lymphopenia in two more studies (Karantanos et al., 2019; Diehl et al., 2017). In a very recent study, Chen et al. analyzed 153 patients with solid tumors treated with radiotherapy and ICIs (Chen et al., 2020a). Low lymphocyte counts were linked with low rates of abscopal effects (3.9 % vs. 34.2 %) and significantly poorer progression-free and overall survival.

These findings bring forward sound questions regarding the combination of immunotherapy with radiotherapy since protection from radiation-induced lymphopenia emerges as a critical factor for the clinical substantiation of benefits. It is worthwhile to carefully study the Keynote-001 phase I study results, where 97 patients with progressive locally advanced or metastatic NSCLC were treated with pembrolizumab (Shaverdian et al., 2017). Forty-three % of them had received radiotherapy before the first cycle of immunotherapy. Despite the postulated radiation-induced lymphopenia, this subgroup of patients had a significantly better progression-free and overall survival, suggesting a robust radio-vaccination effect of pre-immunotherapy radiotherapy. This study's long-term analysis reports a higher than 25 % 5-year survival in patients with high PD-L1 expression (Garon et al., 2019), which suggests that we may face a surprising reality where advanced lung cancer is becoming a curable disease. Radiotherapy-induced lymphopenia and radio-vaccination are two contrasting and critical faces of radiotherapy that, once considered and successfully

dealt, will optimize the curability rates offered by immuno-radiotherapy protocols.

4.6. Protection against RT-induced lymphopenia: RT-schedules

Avoidance of lymphopenia during radiotherapy and, especially, during immuno-radiotherapy appears, therefore, as a critical factor that defines treatment outcome. Location and size of radiotherapy fields are strong determinants of lymphotoxicity. This is well presented in a recent review by Ellsworth SG, in 2018, summarizing and showing the experience from a large number of studies (Ellsworth, 2018). A recent study focusing on pelvic irradiation for prostate cancer showed that large radiotherapy fields to include the pelvic lymph nodes resulted in the reduction of lymphocyte counts below 1000/μL in 61 % of patients, compared to 26 % of patients receiving localized radiotherapy (Schad et al., 2019).

The fractionation of radiotherapy and the techniques applied also seem to be critical (Table 1). This became first evident in 1978, when MacLennan et al. published a study in children treated with prophylactic cranial irradiation for acute lymphocytic leukemia, using a range of 5–15 fractions, showing that for each fraction, a 5–6% reduction of lymphocyte counts occurred (MacLennan and Kay, 1978). Reduction of the number of fractions and days of radiotherapy may, therefore, reduce lymphotoxicity, as circulating blood lymphocytes are exposed to radiation for fewer days. This brings forward an eventual advantage of hypofractionation of radiotherapy as a lymphocyte sparing regimen. This hypothesis was verified in a study by Crocenzi et al. comparing the effect of conventionally fractionated (50.4 Gy in 28 fractions) vs. hypofractionated (30 Gy in 10 fractions) neoadjuvant chemoradiotherapy, in patients with pancreatic cancer (Crocenzi et al., 2016). Conventional radiotherapy produced a profound lymphopenia and reduction of T-cells (both CD4+ and CD8+), and this effect was sustained for at least six months. Hypofractionated chemo-radiotherapy induced a mild decrease of T-cells and faster recovery, a result that was independent of treatment volumes.

Reduction of the overall radiotherapy treatment-time, allowed by hypofractionation, seems to have an important role in the protection against lymphopenia in non-small cell lung cancer. Zhao et al. noted that severe lymphopenia (counts <500/μL) occurs at the 5th week of radiotherapy and is linked with poor prognosis. Reduction of the treatment time to 4 weeks using hypofractionated radiotherapy significantly lowered the risk of severe lymphopenia (Zhao et al., 2019). In breast cancer patients receiving postoperative radiotherapy, the development of lymphopenia is a frequent event. Sun et al. showed that patients receiving a 13 fraction hypofractionated accelerated radiotherapy scheme exhibited less lymphopenia compared to patients receiving conventionally fractionated radiotherapy (Sun et al., 2020). The 5-year progression-free survival was better in patients with less prominent lymphopenia. In another study, Yuan et al. observed similar results regarding the intensity of lymphocytopenia, favoring the short radiotherapy regimen. Authors found a prolonged suppression of CD8+ cytotoxic T-cells and B-cells in the conventional radiotherapy group of patients (Yuan and Wang, 2018).

The optimal way to deliver hypofractionated radiotherapy is Stereotactic radiotherapy (SBRT). Among other advantages, SBRT is linked

Table 1
Studies comparing the effects of RT fractionation and techniques on circulating lymphocytes.

Author/year/ reference	No pts	Disease	Site of irradiation	RT Technique	Findings
MacLennan (1978) (MacLennan and Kay, 1978)	41	Leukemia	Cranial	24 Gy in different fractionations (CRT vs. HypoRT)	Increasing drop of LC by 5–6% per one excess fraction
Crocenzi (2016) (Crocenzi et al., 2016)	20	Pancreatic cancer	Upper abdomen	CRT (50.4 Gy in 28f) vs. HypoRT (30 Gy in 10f)	Compared to Hypo RT, CRT induced profound and prolonged drop of LC and of T-cell subsets. The difference was independent of treatment volumes.
Zhao (2019) (Zhao et al., 2019)	115	Non-small cell lung cancer	Chest	Chemo-RT with 2–3 Gy/f for 30–20f	HypoRT was linked with low risk of SL and better prognosis
Sun (2020) (Sun et al., 2020)	598	Breast cancer	Chest (post-operative)	CRT (2 Gyx 25f, 5 w) vs. HypoRT (2.9 Gy x15f, 3 w)	SL is lower in HypoRT treated pts. SL is linked with poor prognosis
Yuan (2018) (Yuan and Wang, 2018)	60	Breast cancer	Chest (post-operative)	CRT (2 Gyx 25f, 5 w) vs. HypoRT (3.1 Gy x13f, 3 w)	HypoRT induced less severe lymphopenia. CRT induced prolonged suppression of CD8+ T-cells and B-cells.
Wild (2016) (Brizel et al., 2000)	133	Pancreatic cancer	Upper abdomen	SBRT (11 Gy x3f) vs. CRT (1.8 Gy x 28f)	5.5 fold increased incidence of SL in pts treated with CRT. SL is linked with poor prognosis.
Byun (2019) (Byun et al., 2019)	920	Hepatocellular cancer	Upper abdomen	SBRT (12–15 Gy x4f) vs. CRT (2 Gy x26-30f + IMRT boost)	SBRT had reduced risk of SL
Zhang (2019) (Zhang et al., 2019)	184	Hepatocellular cancer	Upper abdomen	SBRT (6–10 Gy/f, 79–119 Gy td) vs. HypoRT (>2.5 Gy/f, 58–91 Gy td) vs. CRT (1.8–2.5 Gy/f, 50–84 Gy td)	Higher number of fractions related with SL. complete and partial response rates
Chen (2020) (Chen et al., 2020b)	165	Non-small cell lung cancer	Chest	SBRT (12.5 Gy/f x4f or 6 Gy/f x10f) vs. HypoRT (3 Gy/f x 15f)	V5 was directly linked with SL.
Routtman (2019) (Routtman et al., 2019)	184	Esophageal cancer	Chest	Protons vs. CRT (1.8–2 Gy/f, 23–25f)	Proton therapy was linked with significantly lower incidence of SL.
Shiraishi (2018) (Shiraishi et al., 2018)	272	Esophageal cancer	Chest	Protons vs. X-rays (1.8 Gy/f x28f)	Protons were linked with a 2-fold decreased incidence of SL.
Liu (2020) (Momm et al., 2001)	84	Medulloblastoma	Craniospinal	Protons vs. X-rays (39.6 Gy td)	Protons induced a significantly lower hematological toxicity, including SL.
Mohan (2020) (Mohan et al., 2020)	84	Glioblastoma	Cranial	Protons vs. X-rays	Protons protected against SL due to reduced brain V20

Abbreviations: RT= radiotherapy; pts=patients; CRT= conventional radiotherapy; HypoRT=hypofractionated radiotherapy; f=fraction; w=week; LC=lymphocyte counts; SL=severe lymphopenia; td=total radiation dose; V5=lung volume receiving 5Gy; V20=brain volume receiving 20Gy; SBRT = stereotactic body radiation therapy.

with lower rates of lymphopenia. Wild et al. reported a series of locally advanced pancreatic cancer treated with conventional (50.4 Gy in 28 fractions) vs. SBRT (33 Gy in 5 fractions). One month after radiotherapy, severe lymphopenia (counts <500/μl) was noted in 71.7 % of patients treated with conventional radiotherapy vs. 13.8 % in patients receiving SBRT (Wild et al., 2016). Of interest, lymphopenia was significantly related to poor survival regardless of the schedule of radiotherapy. SBRT radiotherapy for hepatocellular cancer (4 fractions of 12–15 Gy) was significantly linked with a lower risk of severe lymphopenia compared to a 60 Gy conventionally fractionated radiotherapy (Byun et al., 2019). Similarly, Zhang et al. also found an important sparing effect of SBRT on lymphocyte counts compared to conventionally fractionated radiotherapy in patients with hepatocellular cancer (Zhang et al., 2019). A phase 2 study on the combination of nivolumab with stereotactic radiotherapy (8 Gy x 3 fractions) in 20 patients with advanced melanoma showed that lymphopenia was minimal, as noted in only 3/20 patients (Sundahl et al., 2019). A study by Chen et al. in patients with NSCLC receiving SBRT or 3 Gy/day fractionation showed that the V5 (lung volume reiving 5 Gy) was directly related to lymphopenia only in patients receiving conventional radiotherapy (Chen et al., 2020b).

It is becoming evident that the lymphatic tissue should be considered as an organ at risk for the planning of radiotherapy and the choice of radiotherapy schedules, as lymphopenia is related to poor survival in patients receiving radiotherapy, chemo-radiotherapy, or immuno-radiotherapy. Hypofractionation and acceleration of radiotherapy, especially when SBRT is applied, may significantly reduce the risk, nadir, and lymphopenia duration.

4.7. Protection against RT-induced lymphopenia: particle RT

Proton radiotherapy seems to produce much lower lymphopenia (Table 1). The 'Bragg peak' effect allows an important reduction of the dose delivered to the blood circulating in irradiated normal tissues and the bone marrow. Moreover, proton or heavy ion radiotherapy is by definition a hypofractionated radiotherapy scheme delivered within a small number of fractions. Routman et al. reported a significantly reduced incidence of grade 4 lymphopenia in patients with esophageal cancer treated with proton radiotherapy (24 % incidence) as compared to patients receiving conventional X-ray radiotherapy (56 %) (Routman et al., 2019). Similar results with reduced incidence of grade 4 lymphopenia (17.6 % vs. 40.4 %) in esophageal cancer patients treated with proton therapy have been reported by Shiraishi et al. (Shiraishi et al. (2018)). In patients with medulloblastoma or glioblastoma, proton therapy was linked with significantly higher lymphocyte counts throughout treatment than X-ray radiotherapy (Liu et al., 2020; Mohan et al., 2020).

4.8. Protection against RT/chemotherapy-induced lymphopenia: cytoprotection

Amifostine (WR2721) is the only agent approved for the protection of cancer patients undergoing radiotherapy for head-neck cancer or treated with cisplatin chemotherapy (Koukourakis, 2002). Unfortunately, the large randomized studies focus on the protective effects of amifostine against xerostomia, neutropenia, anemia, and platelet toxicity and do not report on the effect on lymphocytes (Brizel et al., 2000; Kemp et al., 1996). Amifostine stimulates bone marrow in patients with myelodysplastic disease (Schanz et al., 2009). Experimental studies suggest a strong effect of amifostine in protecting lymphocytes against cytogenetic damage and apoptosis induced by radiation (Müller et al., 2004; Saavedra et al., 2010).

Clinical studies have also provided important data. Momm et al. randomized 38 patients with head-neck cancer to receive radiotherapy with or without amifostine. Control patients experienced a decrease of leukocyte counts than amifostine treated patients, but this seemed to be mainly due to the protection of granulopoiesis (Momm et al., 2001). A study of ours in breast cancer patients treated with postoperative hypofractionated radiotherapy, with or without amifostine, reported significant protection of CD4, CD8, CD19, and CD56 subtypes (Koukourakis et al., 2003). Moreover, amifostine induced expansion of CD95/Fas and CD31 expressing lymphocytes. In another study, we recorded an enhanced recovery of T-cell and NK-cells in patients receiving amifostine during radiotherapy for head-neck cancer (Koukourakis et al., 2009).

A low dose of amifostine has also been found to protect lymphocyte DNA against cisplatin, irinotecan, mitomycin, and anthracyclins (Prieto González et al., 2009; Lialiaris et al., 2009; Camelo et al., 2008; Wozniak et al., 2008; Błasiak et al., 2002). Provinivalli et al. studied 19 patients with advanced gynecological cancers treated with neoadjuvant chemotherapy with cisplatin, adriamycin, and cyclophosphamide (Provinciali et al., 1999). Using flow-cytometry analysis of peripheral blood lymphocytes, the authors showed that pre-treatment with amifostine prevented the apoptosis of PBLs induced by drugs.

The clinical significance of amifostine protection effects on the immune system or eventual immunostimulatory activities demands a thorough re-evaluation. Re-analysis of large randomized trials performed in the past, focusing on lymphotoxicity, may provide important evidence.

4.9. Immuno-fertilization of the tumor microenvironment

Further to the systemic lymphocytic abundance, the intratumoral entrance and accumulation of lymphocytes are of equal importance. Impaired tumor vascularity, poor blood flow due to immature vessels, and hypoxic/acidic metabolic conditions prevailing in the tumor microenvironment raise a barrier to the colonization of the tumor stroma by lymphocytes (Koukourakis and Giatromanolaki, 2020). In contrast to normal tissues with an overall neutral extracellular microenvironment, tumors are often acidic with a pH between 6.5 and 6.9 (Vaupel et al., 1989). Anaerobic glycolysis mediated by lactate dehydrogenase LDHA activity and overexpression of carbonic anhydrase CAIX is considered the main causes of enrichment of the extracellular matrix with protons (Swietach et al., 2014). Hypoxia and acidity are potent suppressors of the anti-tumor immune response, blocking cytotoxic cell activity, and proliferation (Noman et al., 2015). As the Hypoxia Inducible Factor HIF1α transcriptionally regulates both LDHA and CAIX, HIF-targeting agents are expected to have a role in the immuno-fertilization of the tumor microenvironment (Wigerup et al., 2016).

For the time being, the only therapy available to target microenvironment conditions is the alkalinization of the intratumoral pH through sodium bicarbonate. Intraperitoneal administration of sodium bicarbonate in experimental animals increases intratumoral pH by 0.6 pH units (Raghunand et al., 2001). Robey et al. showed that oral administration of sodium bicarbonate selectively increases intratumoral pH and reduces tumor metastatization (Robey et al., 2009). A dose of 0.18 g/kg/day was sufficient to counteract the acidic load of some mg's small tumors. The administration of sodium bicarbonate in cancer patients has been used for the urine alkalization for the prevention of nephrotoxicity of high-dose methotrexate chemotherapy (HDMTX). Intravenous administration of sodium bicarbonate (75–150mEq/L iv continuous infusion at 175 mL/h) to reach a urine pH of 7 is the goal before administering HDMTX. An oral regimen of 2600 mg (4 tablets of 650 mg) 6-times daily (or a median of 66.4mEq/m/day) has been also applied (Reed et al., 2019; Visage et al., 2019). In a study by Hamaguchi et al., oral bicarbonate 3–5 g/day was administered to increase urine pH above 7.0 in patients with pancreatic cancer treated with

chemotherapy, showing a better outcome due to alkalinization of the tumor microenvironment and higher chemotherapy efficacy (Hamaguchi et al., 2020). Systemic alkalosis is expected to occur only at very high doses exceeding 0.5 g/kg/day (Martin et al., 2012). Doses up to 12.6 g per day for 46 days have been safely administered (Lemann et al., 1965).

4.10. Selective stimulation of cytotoxic lymphocyte proliferation: Cytokines

Further to lymphocyte protection, policies that promote lymphocyte proliferation, especially when these favor cytotoxic over regulatory cells, may be of important therapeutic value. Several cytokines are involved in the proliferation and survival of lymphocytes. Interleukin-2 (IL2) binds to specific receptors on CD4+ and CD8+ lymphocytes (e.g. IL-2Rα/CD25, IL-2Rβ/CD122, and IL-2Rγ/CD132) inducing proliferation (Boyman and Sprent, 2012). The major problem is that binding to IL-Rα, IL2, promotes the proliferation of regulatory CD4 lymphocytes, which may be detrimental for any immunotherapy. Mutant molecules of IL-2 have been designed (IL-2v) that bind to receptors β and γ, avoiding expansion of Tregs (Heaton et al., 1993b).

IL-15 also binds to CD132/CD122 and its specific receptor IL-15Rα. IL-15 is mainly produced by stroma cells and monocytes and promotes memory CD8+ T-cell and NK cell survival (Conlon et al., 2015). However, it seems also to induce immune checkpoint inhibitory molecules, so that its role in immunotherapy is questionable. IL-15 agonist molecules are in clinical trials (Knudson et al., 2020).

IL-7 binds to a heterodimer that consists of IL-7Rα/CD127 and CD132 and is essential for T-cell generation and differentiation. This is produced by thymic stroma cells, follicular dendritic cells, and epithelial cells (Alpdogan and van den Brink, 2005). rhIL-7 administration increases CD4+ and CD8+ T-cell counts in cancer patients (Tredan et al., 2015). An initial phase of T-cell depletion from the peripheral blood due to the rapid distribution of T-cells in tissues is noted (Rosenberg et al., 2006). IL-7 seems a most promising cytokine to support lymphocyte abundance during chemotherapy in cancer patients (Merchant et al., 2016).

4.11. Selective stimulation of cytotoxic lymphocyte proliferation: active specific immunotherapy

Active specific immunotherapy (ASI) is an interesting immunotherapy policy, extensively studied during the past decades. Heavily irradiated, unable to proliferate, autologous cancer cells have been used as a vaccine to stimulate anti-tumor immunity since the early '20 s (Kellock et al., 1922). Following a large number of positive phase I/II trials, two randomized trials published in 1999–2000 on colorectal cancer showed significant improvement of overall survival when ASI was applied as a postoperative adjuvant regimen (Vermorken et al., 1999; Harris et al., 2000). Due to the introduction of IFNs and IL2, clinical research lost its focus on ASI. Nevertheless, cancer cell vaccines engineered to secrete cytokines like GM-CSF or vaccines produced by the fusion of irradiated cancer cells with dendritic cells remains a hot area of research (Lutz et al., 2011; Wei et al., 2006). Such radio-induced vaccines, delivered transdermally or intra-lymphatically, may stimulate individual cancer-specific cytotoxic immune responses in distant lymph nodes, as regional lymph nodes are mostly destroyed by radiation. Expansion of cytotoxic T-cell and macrophage subpopulations is expected to occur. The blockage of immune checkpoint molecules would maximize ASI activity in both in-field and out-field radiotherapy areas.

4.12. Selective depletion of immunosuppressive cells

Another exciting policy is targeting intratumoral immunosuppressive/regulatory T-cells (T*regs*) or of Myeloid-Derived Suppressor Cells (MDSCs). These later are immature myeloid cells generated in cancer patients' bone marrow that fail to enter a terminal differentiation (Gabrilovich and Nagaraj, 2009). Such cells abrogate the cytotoxic activity of T-cells and NK-cells. Accumulation of T*regs* in the tumor stroma has been linked with poor prognosis in many human carcinomas, like colorectal and breast cancer (Kuwahara et al., 2019; Wang et al., 2019). At least at an experimental level, there are several approaches that could reduce the presence of T*regs* in the tumor microenvironment. T*regs* are more sensitive to cyclophosphamide treatment than cytotoxic T-cells (Heylmann et al., 2013). A possible explanation of this differential sensitivity of T*regs* to cyclophosphamide is that they lack expression of ATP-binding cassette (ABC) transporters, present in effector T-cells. Thus, T*regs* ability to extrude drugs, like cyclophosphamide, is impaired (Dimeloe et al., 2014). Metronomic treatment with cyclophosphamide selectively damages T*regs* and promotes anti-tumor T-cell immunity (Ghiringhelli et al., 2007). Huijts et al. found that low dose daily administration of cyclophosphamide and an mTOR inhibitor depletes T*regs* and MDSCs from renal cell carcinomas without affecting the presence of cytotoxic CD8+ T-cells (Huijts et al., 2019).

Infiltration of tumors by T*regs* enhances resistance to radiotherapy and anti-PD-1 immunotherapy (Oweida et al., 2018). Administration of anti-CD25 monoclonal antibodies, directed against the CD25 antigen characterizing CD4+T*regs*, restores tumor sensitivity to immunotherapy. In an interesting experimental study, Son et al. noted that the combination of radiotherapy with low-dose cyclophosphamide or anti-CD25 antibodies decreases T*regs* in the spleen and the tumors and enhances effector T-cell response (Son et al., 2015). These studies are interesting as anti-CD25 antibodies, like daclizumab, approved for the treatment of multiple sclerosis and transplant rejection, are available in the clinical practice. Administration of daclizumab, in melanoma patients depletes CD4+Foxp3+CD25+ regulatory T-cells in the peripheral blood (Jacobs et al., 2010).

Thorough research is on ging to target specific molecules, like OX40, GITR, or ICOS, expressed by T*regs* aiming to repress Treg function and enhance anti-tumor immune response (Kim et al., 2020). Inhibition of MDSC activity also has focused attention. Inhibitors of phosphodiesterase-5 by sildenafil and tadalafil downregulate arginase and iNOS activity in MDSC, blocking their immunosuppressive activity (Serafini et al., 2006; Weed et al., 2015). Blocking chemokine CXCR2 and CXCR5 activity with specific antagonists is also a promising tool to abrogate MDSC activity in tumors (Sun et al., 2019).

5. Discussion

Clinical experience suggests that radiotherapy should be dealt with as a double-edged sword in the current immunotherapy era. Cancer damage induced by radiation promotes radio-vaccination, enhancing the recognition of tumor cells by dendritic cells and triggering a T-cell cytotoxic response that, in turn, augments local and abscopal immunotherapy activity. In parallel to the above beneficial effect, radiotherapy induces intense and prolonged lymphopenia, reducing the number of cytotoxic lymphocytes that may reach the tumor microenvironment. As immuno-radiotherapy is gradually emerging as a most promising therapeutic approach, two contrasting concepts should be meticulously dealt with when designing clinical trials. Hypofractionated SBRT and accelerated radiotherapy seem to be the best option for combinations with immunotherapy. These have a more potent radio-vaccination ability and, they also produce less severe and short-lasting lymphopenia. Cytoprotective agents, stimulatory agents specific for cytotoxic cells, and agents blocking regulatory cell activity are expected to become potent radiotherapy allies in combination with immune checkpoint inhibitors. It is also suggested that the lymphocyte status (systemic and intratumoral) of patients recruited in immunotherapy trials should focus attention. Easily assessable lymphocyte markers may

prove extremely important in selecting patients for immunotherapy or pre-immunotherapy interventions to restore peripheral and intra-tumoral lymphopenia.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Ahmadzadeh, M., Rosenberg, S.A., 2006. IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients. Blood 107, 2409–2414.

Alpdogan, O., van den Brink, M.R., 2005. IL-7 and IL-15: therapeutic cytokines for immunodeficiency. Trends Immunol. 26, 56–64.

Asai, T., Inaba, S., Ohto, H., Osada, K., Suzuki, G., Takahashi, K., Tadokoro, K., Minami, M., 2000. Guidelines for irradiation of blood and blood components to prevent post-transfusion graft-vs.-host disease in Japan. Transfus. Med. 10, 315–320.

Bambury, R.M., Teo, M.Y., Power, D.G., et al., 2013. The association of pre-treatment neutrophil to lymphocyte ratio with overall survival in patients with glioblastoma multiforme. J. Neurooncol. 114, 149–154.

Bashford, E.F., Murray, J.A., Cramer, W., 1907. The natural and induced resistance of mice to the growth of cancer. Proc. R. Soc. B. doi:10.1098/rspb.1907.0014.

Berczi, I., Strausbauch, P., Sehon, A.H., 1973. Rejection of tumor cells in vitro. Science 180, 1289–1291.

Bhethanabhotla, S., Jain, S., Kapoor, G., Mahajan, A., Chopra, A., Vishnubhatla, S., Bakhshi, S., 2017. Outcome of pediatric advanced Hodgkin lymphoma treated with ABVD and predictors of inferior survival: a multicenter study of 186 patients. Leuk. Lymphoma 58, 1617–1623.

Bigot, F., Castanon, E., Baldini, C., Hollebecque, A., Carmona, A., Postel-Vinay, S., Angevin, E., Armand, J.P., Ribrag, V., Aspeslagh, S., Varga, A., Bahleda, R., Menis, J., Gazzah, A., Michot, J.M., Marabelle, A., Soria, J.C., Massard, C., 2017. Prospective validation of a prognostic score for patients in immunotherapy phase I trials: the Gustave Roussy Immune Score (GRIm-Score). Eur. J. Cancer 84, 212–218.

Błasiak, J., Gloc, E., Młynarski, W., Drzewoski, J., Skórski, T., 2002. Amifostine differentially modulates DNA damage evoked by idarubicin in normal and leukemic cells. Leuk. Res. 26, 1093–1096.

Blomgren, H., Wasserman, J., Rotstein, S., Petrini, B., von Stedingk, L.V., 1988. Impact of adjuvant chemotherapy on spontaneous and poke week mitogen triggered immunoglobulin secretion of blood lymphocytes in operable breast cancer. Anticancer Res. 8, 839–844.

Borghaei, H., Paz-Ares, L., Horn, L., et al., 2015. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N. Engl. J. Med. 373, 1627–1639.

Boyman, O., Sprent, J., 2012. The role of interleukin-2 during homeostasis and activation of the immune system. Nat. Rev. Immunol. 12, 180–190.

Brizel, D.M., Wasserman, T.H., Henke, M., Strnad, V., Rudat, V., Monnier, A., Eschwege, F., Zhang, J., Russell, L., Oster, W., Sauer, R., 2000. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. J. Clin. Oncol. 18, 3339–3345.

Brooks, W.H., Netsky, M.G., Normansell, D.E., Horwitz, D.A., 1972. Depressed cell-mediated immunity in patients with primary intracranial tumors. Characterization of a humoral immunosuppressive factor. J. Exp. Med. 136, 1631–1647.

Byun, H.K., Kim, N., Park, S., Seong, J., 2019. Acute severe lymphopenia by radiotherapy is associated with reduced overall survival in hepatocellular carcinoma. Strahlenther. Onkol. 195, 1007–1017.

Camelo, R.M., Kehdy, F.S., Salas, C.E., Lopes, M.T., 2008. Amifostine protection against mitomycin-induced chromosomal breakage in fanconi anaemia lymphocytes. Molecules 13, 1759–1772.

Cézé, N., Thibault, G., Goujon, G., et al., 2011. Pre-treatment lymphopenia as a prognostic biomarker in colorectal cancer patients receiving chemotherapy. Cancer Chemother. Pharmacol. 68, 1305–1313.

Chadha, A.S., Liu, G., Chen, H.C., Das, P., Minsky, B.D., Mahmood, U., Delclos, M.E., Suh, Y., Sawakuchi, G.O., Beddar, S., Katz, M.H., Fleming, J.B., Javle, M.M., Varadhachary, G.R., Wolff, R.A., Crane, C.H., Wang, X., Thames, H., Krishnan, S., 2017. Does unintentional splenic radiation predict outcomes after pancreatic cancer radiation therapy? Int. J. Radiat. Oncol. Biol. Phys. 97, 323–332.

Che, Y.Q., Zhang, Y., Wang, D., Liu, H.Y., Shen, D., Luo, Y., 2019. Baseline lymphopenia: a predictor of poor outcomes in HER2 positive metastatic breast cancer treated with trastuzumab. Drug Des. Devel. Ther. 13, 3727–3734.

Chen, W., 2011. Tregs in immunotherapy: opportunities and challenges. Immunotherapy 3, 911–914.

Chen, D., Verma, V., Patel, R.R., Barsoumian, H.B., Cortez, M.A., Welsh, J.W., 2020a. Absolute lymphocyte count predicts abscopal responses and outcomes in patients receiving combined immunotherapy and radiotherapy: a prospective-retrospective analysis of 3 phase I/II trials. Int. J. Radiat. Oncol. Biol. Phys. pii: S0360-3016:30158-30159.

Chen, D., Patel, R.R., Verma, V., Ramapriyan, R., Barsoumian, H.B., Cortez, M.A., Welsh, J.W., 2020b. Interaction between lymphopenia, radiotherapy technique, dosimetry, and survival outcomes in lung cancer patients receiving combined immunotherapy and radiotherapy. Radiother. Oncol. 150, 114–120.

Cho, Y., Park, S., Byun, H.K., Lee, C.G., Cho, J., Hong, M.H., Kim, H.R., Cho, B.C., Kim, S., Park, J., Yoon, H.I., 2019. Impact of treatment-related lymphopenia on immunotherapy for advanced non-small cell lung cancer. Int. J. Radiat. Oncol. Biol. Phys. 105, 1065–1073.

Conlon, K.C., Lugli, E., Welles, H.C., Rosenberg, S.A., Fojo, A.T., Morris, J.C., et al., 2015. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J. Clin. Oncol. 33, 74–82.

Costello, R.T., Gastaut, J.A., Olive, D., 1999. Tumor escape from immune surveillance. Arch. Immunol. Ther. Exp. (Warsz.) 47, 83–88.

Crocenzi, T., Cottam, B., Newell, P., Wolf, R.F., Hansen, P.D., Hammill, C., Solhjem, M.C., To, Y.Y., Greathouse, A., Tormoen, G., Jutric, Z., Young, K., Bahjat, K.S., Gough, M.J., Crittenden, M.R., 2016. A hypofractionated radiation regimen avoids the lymphopenia associated with neoadjuvant chemoradiation therapy of borderline resectable and locally advanced pancreatic adenocarcinoma. J. Immunother. Cancer 4, 45.

Diehl, A., Yarchoan, M., Hopkins, A., Jaffee, E., Grossman, S.A., 2017. Relationships between lymphocyte counts and treatment-related toxicities and clinical responses in patients with solid tumors treated with PD-1 checkpoint inhibitors. Oncotarget 8, 114268–114280.

Dimeloe, S., Frick, C., Fischer, M., Gubser, P.M., Razik, L., Bantug, G.R., Ravon, M., Langenkamp, A., Hess, C., 2014. Human regulatory T cells lack the cyclophosphamide-extruding transporter ABCB1 and are more susceptible to cyclophosphamide-induced apoptosis. Eur. J. Immunol. 44 (December 12), 3614–3620. doi:10.1002/eji.201444879.

Dyck, L., Mills, K.H.G., 2017. Immune checkpoints and their inhibition in cancer and infectious diseases. Eur. J. Immunol. 47, 765–779.

Ellsworth, S.G., 2018. Field size effects on the risk and severity of treatment-induced lymphopenia in patients undergoing radiation therapy for solid tumors. Adv. Radiat. Oncol. 3, 512–519.

Gabrilovich, D., Nagaraj, S., 2009. Myeloid-derived-suppressor cells as regulators of the immune system. Nat. Rev. Immunol. 9, 162–174.

Garcia-Aguilar, J., Chow, O.S., Smith, D.D., Marcet, J.E., Cataldo, P.A., Varma, M.G., Kumar, A.S., Oommen, S., Coutsoftides, T., Hunt, S.R., Stamos, M.J., Ternent, C.A., Herzig, D.O., Fichera, A., Polite, B.N., Dietz, D.W., Patil, S., Avila, K., 2015. Timing of rectal cancer response to chemoradiation consortium. Effect of adding mFOLFOX6 after neoadjuvant chemoradiation in locally advanced rectal cancer: a multicentre, phase 2 trial. Lancet Oncol. 16, 957–966.

Garon, E.B., Hellmann, M.D., Rizvi, N.A., Carcereny, E., Leighl, N.B., Ahn, M.J., Eder, J.P., Balmanoukian, A.S., Aggarwal, C., Horn, L., Patnaik, A., Gubens, M., Ramalingam, S.S., Felip, E., Goldman, J.W., Scalzo, C., Jensen, E., Kush, D.A., Hui, R., 2019. Five-year overall survival for patients with advanced non–small-cell lung cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study. J. Clin. Oncol. 37, 2518–2527.

Ghiringhelli, F., Menard, C., Puig, P.E., Ladoire, S., Roux, S., Martin, F., et al., 2007. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end-stage cancer patients. Cancer Immunol. Immunother. 56, 641–648.

Giatromanolaki, A., Koukourakis, I.M., Balaska, K., Mitrakas, A.G., Harris, A.L., Koukourakis, I.M., 2019. Programmed death-1 receptor (PD-1) and PD-ligand-1 (PD-L1) expression in non-small cell lung cancer and the immune-suppressive effect of anaerobic glycolysis. Med. Oncol. 36, 76.

Guevremont, C., Alasker, A., Karakiewicz, P.I., 2009. Management of sorafenib, sunitinib, and temsirolimus toxicity in metastatic renal cell carcinoma. Curr. Opin. Support. Palliat. Care 3, 170–179.

Guo, J., Huang, Y., Zhang, X., Zhou, F., Sun, Y., Qin, S., Ye, Z., Wang, H., Jappe, A., Straub, P., Pirotta, N., Gogov, S., 2013. Safety and efficacy of everolimus in Chinese patients with metastatic renal cell carcinoma resistant to vascular endothelial growth factor receptor-tyrosine kinase inhibitor therapy: an open-label phase 1b study. BMC Cancer 13, 136.

Hamaguchi, R., Narui, R., Wada, H., 2020. Effects of alkalization therapy on chemotherapy outcomes in metastatic or recurrent pancreatic cancer. Anticancer Res. 40, 873–880.

Harisiadis, L., Kopelson, G., Chang, C.H., 1977. Lymphopenia caused by cranial irradiation in children receiving craniospinal radiotherapy. Cancer 40, 1102–1108.

Harris, J.E., Ryan, L., Hoover, H.C., Jr., Stuart, R.K., Oken, M.M., Benson, A.B., 3rd, Mansour, E., Haller, D.G., Manola, J., Hanna, M.G., Jr., 2000. Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. J. Clin. Oncol. 18, 148–157.

Heaton, K.M., Ju, G., Grimm, E.A., 1993a. Human interleukin 2 analogs that preferentially bind the intermediate-affinity interleukin 2 receptor lead to reduced secondary cytokine secretion: implications for the use of these interleukin 2 analogs in cancer immunotherapy. Cancer Res. 53, 2597–2602.

Heaton, K.M., Ju, G., Grimm, E.A., 1993b. Human interleukin 2 analogs that preferentially bind the intermediate-affinity interleukin 2 receptor lead to reduced secondary cytokine secretion: implications for the use of these interleukin 2 analogs in cancer immunotherapy. Cancer Res. 53, 2597–2600.

Hensley, M.L., Larkin, J., Fury, M., Gerst, S., Tai, D.F., Sabbatini, P., Konner, J., Orlando, M., Goss, T.L., Aghajanian, C.A., 2008. A phase I trial of pemetrexed plus gemcitabine, given biweekly with B-vitamin support in solid tumor malignancies or advanced epithelial ovarian cancer. Clin. Cancer Res. 14, 6310–6316.

Heylmann, D., Bauer, M., Becker, H., van Gool, S., Bacher, N., Steinbrink, K., Kaina, B., 2013. Human CD4+CD25+ regulatory T cells are sensitive to low dose cyclophosphamide: implications for the immune response. PLoS One 8, e83384.

Ho, C., Sangha, R., Beckett, L., Tanaka, M., Lau, D.H., Eisen, D.B., Burich, R.A., Luciw, P., Khan, I., Mack, P.C., Gandara, D.R., Davies, A.M., 2011. Escalating weekly doses of cetuximab and correlation with skin toxicity: a phase I study. Invest. New Drugs 29, 680–687.

Ho, W.J., Yarchoan, M., Hopkins, A., Mehra, R., Grossman, S., Kang, H., 2018. Association between pre-treatment lymphocyte count and response to PD1 inhibitors in head and neck squamous cell carcinomas. J. Immunother. Cancer 6, 84.

Holub, K., Busato, F., Gouy, S., et al., 2020. Analysis of systemic inflammatory factors and survival outcomes in endometrial cancer patients staged I-III FIGO and treated with postoperative external radiotherapy. J. Clin. Med. 9, 1441.

Huijts, C.M., Lougheed, S.M., Bodalal, Z., van Herpen, C.M., Hamberg, P., Tascilar, M., Haanen, J.B., Verheul, H.M., de Gruijl, T.D., van der Vliet, H.J., Dutch WIN-O Consortium, 2019. The effect of everolimus and low-dose cyclophosphamide on immune cell subsets in patients with metastatic renal cell carcinoma: results from a phase I clinical trial. Cancer Immunol. Immunother. 68, 503–515.

Hurd, E.R., Giuliano, V.J., 1975. The effect of cyclophosphamide on B and T lymphocytes in patients with connective tissue diseases. Arthritis Rheum. 18, 67–75.

Jacobs, J.F., Punt, C.J., Lesterhuis, W.J., Sutmuller, R.P., Brouwer, H.M., Scharenborg, N.M., Klasen, I.S., Hilbrands, L.B., Figdor, C.G., de Vries, I.J., Adema, G.J., 2010. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. Clin. Cancer Res. 16, 5067–5078.

Joseph, N., Dovedi, S.J., Thompson, C., et al., 2016. Pre-treatment lymphocytopaenia is an adverse prognostic biomarker in muscle-invasive and advanced bladder cancer. Ann. Oncol. 27, 294–299.

Kaklamanis, L., Leek, R., Koukourakis, M., Gatter, K.C., Harris, A.L., 1995. Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. Cancer Res. 55, 5191–5194.

Karantanos, T., Karanika, S., Seth, B., Gignac, G., 2019. The absolute lymphocyte count can predict the overall survival of patients with non-small cell lung cancer on nivolumab: a clinical study. Clin. Transl. Oncol. 21, 206–212.

Kellock, T.H., Chambers, H., Russ, S., 1922. An attempt to produce immunity to malignant disease in man. Lancet 5136, 217–219.

Kemp, G., Rose, P., Lurain, J., Berman, M., Manetta, A., Roullet, B., Homesley, H., Belpomme, D., Glick, J., 1996. Amifostine pre-treatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized control trial in patients with advanced ovarian cancer. J. Clin. Oncol. 14, 2101–2112.

Kerbel, R.S., 1974. Mechanisms of tumor-induced immunological deficiencies and their possible significance in relation to the use of immunopotentiators in tumor-bearing hosts. Biomedicine 21, 253–261.

Kim, J.H., Kim, B.S., Lee, S.K., 2020. Regulatory t cells in tumor microenvironment and approach for anticancer immunotherapy. Immune Netw. 20, e4.

Knudson, K.M., Hodge, J.W., Schlom, J., Gameiro, S.R., 2020. Rationale for IL-15 superagonists in cancer immunotherapy. Expert Opin. Biol. Ther. 11 (March), 1–5.

Koizumi, W., Saigenji, K., Ujiie, S., Terashima, M., Sakata, Y., Taguchi, T., Clinical Study Group of Capecitabine, 2003. A pilot phase II study of capecitabine in advanced or recurrent gastric cancer. Oncology 64, 232–236.

Kotsakis, A., Sarra, E., Peraki, M., Koukourakis, M., Apostolaki, S., Souglakos, J., Mavromanomakis, E., Vlachonikolis, J., Georgoulias, V., 2000. Docetaxel-induced lymphopenia in patients with solid tumors: a prospective phenotypic analysis. Cancer 89, 1380–1386.

Kou, F., Lu, Z., Li, J., Zhang, X., Lu, M., Zhou, J., Wang, X., Gong, J., Gao, J., Li, J., Li, Y., Shen, L., 2016. Pretreatment lymphopenia is an easily detectable predictive and prognostic marker in patients with metastatic esophagus squamous cell carcinoma receiving first-line chemotherapy. Cancer Med. 5, 778–786.

Koukourakis, M.I., 2002. Amifostine in clinical oncology: current use and future applications. Anticancer Drugs 13, 181–209.

Koukourakis, M.I., Giatromanolaki, A., 2020. Tumor microenvironment, immune response and post-radiotherapy tumor clearance. Clin. Transl. Oncol. (May), 22.

Koukourakis, M.I., Giatromanolaki, A., Schiza, S., Kakolyris, S., Georgoulias, V., 1999. Concurrent twice-a-week docetaxel and radiotherapy: a dose-escalation trial with immunological toxicity evaluation. Int. J. Radiat. Oncol. Biol. Phys. 43, 107–114.

Koukourakis, M.I., Ktenidou-Kartali, S., Bourikas, G., Kartalis, G., Tsatalas, C., 2003. Amifostine protects lymphocytes during radiotherapy and stimulates the expansion of the CD95/Fas and CD31 expressing T-cells, in breast cancer patients. Cancer Immunol. Immunother. 52, 127–131.

Koukourakis, G.V., Baksevanis, C.N., Zambatis, H., Gritzapis, A., Maltezos, E., Simopoulos, C., Koukourakis, M.I., 2009. Amifostine enhances recovery and expansion of peripheral FAS/CD95+ T- and NK-cell subpopulations during radiotherapy of patients with head-neck cancer. Int. J. Radiat. Biol. 85, 96–104.

Kuo, P., Bratman, S.V., Shultz, D.B., von Eyben, R., Chan, C., Wang, Z., Say, C., Gupta, A., Loo, B.W., Jr., Giaccia, A.J., Koong, A.C., Diehn, M., Le, Q.T., 2014. Galectin-1 mediates radiation-related lymphopenia and attenuates NSCLC radiation response. Clin. Cancer Res. 20, 5558–5569.

Kuwahara, T., Hazama, S., Suzuki, N., Yoshida, S., Tomochika, S., Nakagami, Y., Matsui, H., Shindo, Y., Kanekiyo, S., Tokumitsu, Y., Iida, M., Tsunedomi, R., Takeda, S., Yoshino, S., Okayama, N., Suehiro, Y., Yamasaki, T., Fujita, T., Kawakami, Y., Ueno, T., Nagano, H., 2019. Intratumoural-infiltrating CD4 + and FOXP3 + T cells as strong positive predictive markers for the prognosis of resectable colorectal cancer. Br. J. Cancer 121, 659–665.

Lemann, J., Jr., Lennon, E.J., Goodman, A.D., Litzow, J.R., Relman, A.S., 1965. The net balance of acid in subjects given large loads of acid or alakali. J. Clin. Invest. 44, 507–517.

Lialiaris, T.S., Kotsiou, E., Pouliliou, S., Kareli, D., Makrinou, H., Kouskoukis, A., Papachristou, F., Koukourakis, M., 2009. Cytoprotective activity of amifostine on cultured human lymphocytes exposed to irinotecan. Food Chem. Toxicol. 47, 2445–2449.

Liu, J., Zhao, Q., Deng, W., Lu, J., Xu, X., Wang, R., Li, X., Yue, J., 2017. Radiation-related lymphopenia is associated with spleen irradiation dose during radiotherapy in patients with hepatocellular carcinoma. Radiat. Oncol. 12, 90.

Liu, L.T., Chen, Q.Y., Tang, L.Q., Guo, S.S., Guo, L., Mo, H.Y., Chen, M.Y., Zhao, C., Guo, X., Qian, C.N., Zeng, M.S., Bei, J.X., Tan, J., Chen, S., Hong, M.H., Shao, J.Y., Sun, Y., Ma, J., Mai, H.Q., 2018. The prognostic value of treatment-related lymphopenia in nasopharyngeal carcinoma patients. Cancer Res. Treat. 50, 19–29.

Liu, K.X., Ioakeim-Ioannidou, M., Susko, M.S., Rao, A.D., Yeap, B.Y., Snijders, A.M., Ladra, M.M., Vogel, J., Zaslowe-Dude, C., Marcus, K.J., Yock, T.I., Grassberger, C., Braunstein, S.E., Haas-Kogan, D.A., Terezakis, S.A., MacDonald, S.M., 2020. A multi-institutional comparative analysis of proton and photon therapy-induced hematologic toxicity in patients with medulloblastoma. Int. J. Radiat. Oncol. Biol. Phys. S0360-3016.

Lutz, E., Yeo, C.J., Lillemoe, K.D., Biedrzycki, B., Kobrin, B., Herman, J., Sugar, E., Piantadosi, S., Cameron, J.L., Solt, S., Onners, B., Tartakovsky, I., Choi, M., Sharma, R., Illei, P.B., Hruban, R.H., Abrams, R.A., Le, D., Jaffee, E., Laheru, D., 2011. A lethally irradiated allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. Ann. Surg. 253, 328–335.

MacLennan, I.C., Kay, H.E., 1978. Analysis of treatment in childhood leukemia. IV. The critical association between dose fractionation and immunosuppression induced by cranial irradiation. Cancer 41, 108–111.

Manyam, B.V., Garsa, A.A., Chin, R.I., Reddy, C.A., Gastman, B., Thorstad, W., Yom, S.S., Nussenbaum, B., Wang, S.J., Vidimos, A.T., Koyfman, S.A., 2017. A multi institutional comparison of outcomes of immunosuppressed and immunocompetent patients treated with surgery and radiation therapy for cutaneous squamous cell carcinoma of the head and neck. Cancer 123, 2054–2060.

Martin, N.K., Robey, I.F., Gaffney, E.A., Gillies, R.J., Gatenby, R.A., Maini, P.K., 2012. Predicting the safety and efficacy of buffer therapy to raise tumour pHe: an integrative modelling study. Br. J. Cancer 106, 1280–1287.

Martinet, L., Smyth, M.J., 2015. Balancing natural killer cell activation through paired receptors. Nat. Rev. Immunol. 15, 243–254.

Ménétrier-Caux, C., Ray-Coquard, I., Blay, J.Y., Caux, C., 2019. Lymphopenia in cancer patients and its effects on response to immunotherapy: an opportunity for combination with cytokines? J. Immunother. Cancer 7, 85.

Merchant, M.S., Bernstein, D., Amoako, M., Baird, K., Fleisher, T.A., Morre, M., et al., 2016. Adjuvant immunotherapy to improve outcome in high-risk pediatric sarcomas. Clin. Cancer Res. 22, 3182–3191.

Mohan, R., Liu, A.Y., Brown, P.D., Mahajan, A., Dinh, J., Chung, C., McAvoy, S., McAleer, M.F., Lin, S.H., Li, J., Ghia, A.J., Zhu, C., Sulman, E.P., de Groot, J.F., Heimberger, A.B., McGovern, S.L., Grassberger, C., Shih, H., Ellsworth, S., Grosshans, D.R., 2020. Proton therapy reduces the likelihood of high-grade radiation-induced lymphopenia in glioblastoma patients: phase II randomized study of protons vs. photons. Neuro Oncol. Aug 5:noaa182.

Momm, F., Bechtold, C., Rudat, V., Strnad, V., Tsekos, A., Fischer, K., Henke, M., 2001. Alteration of radiation-induced hematotoxicity by amifostine. Int. J. Radiat. Oncol. Biol. Phys. 51, 947–951.

Moon, H., Roh, J.L., Lee, S.W., et al., 2016. Prognostic value of nutritional and hematologic markers in head and neck squamous cell carcinoma treated by chemoradiotherapy. Radiother. Oncol. 118, 330–334.

Müller, A.C., Pigorsch, S., Beyer, C., Lautenschläger, C., Dunst, J., 2004. Radioprotective effects of amifostine in vitro and in vivo measured with the comet assay. Strahlenther. Onkol. 180 (August), 517–525.

Najarian, T., Miller, A., Zimelman, A.P., Ki Hong, W., 1981. Hematologic effects of cis-platinum-bleomycin therapy. Oncology 38, 195–197.

Ng, S.P., Bahig, H., Jethanandani, A., Pollard, C., Berends, J., Sturgis, E.M., Johnson, F.M., Elgohari, B., Elhalawani, H., Rosenthal, D.I., Skinner, H.D., Gunn, G.B., Phan, J., Frank, S.J., Mohamed, A.S.R., Fuller, C.D., Garden, A.S., 2020. Lymphopenia during radiotherapy in patients with oropharyngeal cancer. Radiother. Oncol. 145, 95–100.

Noman, M.Z., Hasmim, M., Messai, Y., Terry, S., Kieda, C., Janji, B., Chouaib, S., 2015. Hypoxia: a key player in anti-tumor immune response. A review in the theme: cellular responses to hypoxia. Am. J. Physiol., Cell Physiol. 309, C569-79.

O'Donnell, J.S., Teng, M.W.L., Smyth, M.J., 2019. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat. Rev. Clin. Oncol. 16, 151–167.

Oweida, A., Hararah, M.K., Phan, A., Binder, D., Bhatia, S., Lennon, S., Bukkapatnam, S., Van Court, B., Uyanga, N., Darragh, L., Kim, H.M., Raben, D., Tan, A.C., Heasley, L., Clambey, E., Nemenoff, R., Karam, S.D., 2018. Resistance to radiotherapy and PD-L1 blockade is mediated by TIM-3 upregulation and regulatory T-cell infiltration. Clin. Cancer Res. 24, 5368–5380.

Patel, S., Wang, S., Snuderl, M., Karajannis, M.A., 2018. Pre-treatment lymphopenia and indication of tumor-induced systemic immunosuppression in medulloblastoma. J. Neurooncol. 136, 541–544.

Patel, D.A., Xi, J., Luo, J., Hassan, B., Thomas, S., Ma, C.X., Campian, J.L., 2019. Neutrophil-to-lymphocyte ratio as a predictor of survival in patients with triple-negative breast cancer. Breast Cancer Res. Treat. 174, 443–452.

Pike, L.R.G., Bang, A., Mahal, B.A., Taylor, A., Krishnan, M., Spektor, A., Cagney, D.N., Aizer, A.A., Alexander, B.M., Rahma, O., Balboni, T., Ott, P.A., Hodi, F.S., Schoenfeld, J.D., 2019. The impact of radiation therapy on lymphocyte count and survival in metastatic cancer patients receiving PD-1 immune checkpoint inhibitors. Int. J. Radiat. Oncol. Biol. Phys. 103, 142–151.

Pouliliou, S.E., Lialiaris, T.S., Dimitriou, T., Giatromanolaki, A., Papazoglou, D., Pappa, A., Pistevou, K., Kalamida, D., Koukourakis, M.I., 2015. Survival fraction at 2 Gy and γH2AX expression kinetics in peripheral blood lymphocytes from cancer patients: relationship with acute radiation-induced toxicities. Int. J. Radiat. Oncol. Biol. Phys. 92, 667–674.

Prieto González, E.A., Fuchs, A.G., Sánchez, G.S., 2009. Amifostine (WR2721) confers DNA protection to in vivo cisplatin-treated murine peripheral blood leukocytes. Dose Response 7, 234–246.

Provinciali, M., Ciavattini, A., Di Stefano, G., Argentati, K., Garzetti, G.G., 1999. In vivo amifostine (WR-2721) prevents chemotherapy-induced apoptosis of peripheral blood lymphocytes from cancer patients. Life Sci. 64, 1525–1532.

Raghunand, N., Mahoney, B., van Sluis, R., Baggett, B., Gillies, R.J., 2001. Acute metabolic alkalosis enhances the response of C3H mouse mammary tumors to the weak base mitoxantrone. Neoplasia 3, 227–235.

Ramey, S.J., Balise, R., Ritch, C., Punnen, S., Ishkanian, A.S., Pollack, A., Abramowitz, M.C., 2015. Immunocompromised patients with prostate cancer treated with external beam radiation: a matched pair analysis. Int. J. Radiat. Oncol. Biol. Phys. 93 (Suppl. 3), E206.

Ray-Coquard, I., Cropet, C., Van Glabbeke, M., Sebban, C., Le Cesne, A., Judson, I., Tredan, O., Verweij, J., Biron, P., Labidi, I., Guastalla, J.P., Bachelot, T., Perol, D.,

Chabaud, S., Hogendoorn, P.C., Cassier, P., Dufresne, A., Blay, J.Y., European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group, 2009. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. Cancer Res. 69, 5383–5391.

Reed, D.R., Pierce, E.J., Sen, J.M., Keng, M.K., 2019. A prospective study on urine alkalization with an oral regimen consisting of sodium bicarbonate and acetazolamide in patients receiving high-dose methotrexate. Cancer Manag. Res. 11, 8065–8072.

Reismüller, B., Azizi, A.A., Peyrl, A., Heinrich, M., Gruber-Olipitz, M., Luckner, D., Rothschild, K.V., Slavc, I., 2010. Feasibility and tolerability of bevacizumab in children with primary CNS tumors. Pediatr. Blood Cancer 54 (May 5), 681–686.

Robey, I.F., Baggett, B.K., Kirkpatrick, N.D., Roe, D.J., Dosescu, J., Sloane, B.F., Hashim, A.I., Morse, D.L., Raghunand, N., Gatenby, R.A., Gillies, R.J., 2009. Bicarbonate increases tumor pH and inhibits spontaneous metastases. Cancer Res. 69, 2260–2268.

Rosenberg, S.A., Sportes, C., Ahmadzadeh, M., Fry, T.J., Ngo, L.T., Schwarz, S.L., et al., 2006. IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. J Immunother. 29, 313–319.

Routman, D.M., Garant, A., Lester, S.C., Day, C.N., Harmsen, W.S., Sanheuza, C.T., Yoon, H.H., Neben-Wittich, M.A., Martenson, J.A., Haddock, M.G., Hallemeier, C.L., Merrell, K.W., 2019. A comparison of grade 4 lymphopenia with proton versus photon radiation therapy for esophageal cancer. Adv. Radiat. Oncol. 4, 63–69.

Saavedra, M.M., Henríquez-Hernández, L.A., Lara, P.C., Pinar, B., Rodríguez-Gallego, C., Lloret, M., 2010. Amifostine modulates radio-induced apoptosis of peripheral blood lymphocytes in head and neck cancer patients. J. Radiat. Res. 51, 603–607.

Sano, T., Saijo, N., Sasaki, Y., Shinkai, T., Eguchi, K., Tamura, T., Sakurai, M., Takahashi, H., Nakano, H., Nakagawa, K., et al., 1988. Three schedules of recombinant human interleukin-2 in the treatment of malignancy: side effects and immunologic effects in relation to serum level. Jpn. J. Cancer Res. 79, 131–143.

Schad, M.D., Dutta, S.W., Muller, D.M., Wijesooriya, K., Showalter, T.N., 2019. Radiation-related lymphopenia after pelvic nodal irradiation for prostate cancer. Adv. Radiat. Oncol. 4, 323–330.

Schanz, J., Jung, H., Wörmann, B., Gassmann, W., Petersen, T., Hinke, A., Haase, D., 2009. Amifostine has the potential to induce haematologic responses and decelerate disease progression in individual patients with low- and intermediate-1-risk myelodysplastic syndromes. Leuk. Res. 33, 1183–1188.

Schirrmacher, V., Landolfo, S., Zawatzky, R., Kirchner, H., 1981. Immunogenetic studies on the resistance of mice to highly metastatic DBA/2 tumor cell variants. II. Influence of minor histocompatibility antigens on tumor resistance, gamma-interferon induction, and cytotoxic response. Invasion Metastasis 1, 175–194.

Serafini, P., Meckel, K., Kelso, M., Noonan, K., Califano, J., Koch, W., et al., 2006. Phosphodiesterase-5 inhibition augments endogenous anti-tumor immunity by reducing myeloid-derived suppressor cell function. J. Exp. Med. 203, 2691–2702.

Setti, M., Bignardi, D., Ballestrero, A., Ferrando, F., Musselli, C., Blanchi, S., Basso, M., Bosco, O., Balleari, E., Patrone, F., Indiveri, F., 1999. The induction of distinct cytokine cascades correlates with different effects of granulocyte-colony stimulating factor and granulocyte/macrophage-colony-stimulating factor on the lymphocyte compartment in the course of high-dose chemotherapy for breast cancer. Cancer Immunol. Immunother. 48, 287–296.

Shaverdian, N., Lisberg, A.E., Bornazyan, K., Veruttipong, D., Goldman, J.W., Formenti, S.C., Garon, E.B., Lee, P., 2017. Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial. Lancet Oncol. 18, 895–903.

Shiraishi, Y., Fang, P., Xu, C., Song, J., Krishnan, S., Koay, E.J., Mehran, R.J., Hofstetter, W.L., Blum-Murphy, M., Ajani, J.A., Komaki, R., Minsky, B., Mohan, R., Hsu, C.C., Hobbs, B.P., Lin, S.H., 2018. Severe lymphopenia during neoadjuvant chemoradiation for esophageal cancer: a propensity matched analysis of the relative risk of proton versus photon-based radiation therapy. Radiother. Oncol. 128, 154–160.

Simmler, M.C., Bruley-Rosset, M., 1976. Immunodeficiency in cancer patients and immunorestoration. Cancer Immunol. Immunother. 1, 119–126.

Son, C.H., Bae, J.H., Shin, D.Y., Lee, H.R., Jo, W.S., Yang, K., Park, Y.S., 2015. Combination effect of regulatory T-cell depletion and ionizing radiation in mouse models of lung and colon cancer. Int. J. Radiat. Oncol. Biol. Phys. 92, 390–398.

Stansly, P.G., 1963. Immunological tolerance, infective tolerance and virus-induced neoplastic transformation. Nature 198, 110–111.

Stone, H.B., Peters, L.J., Milas, L., 1979. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. J. Natl. Cancer Inst. 63, 1229–1235.

Suit, H.D., Kastelan, A., 1970. Immunologic status of host and response of a methylcholanthrene-induced sarcoma to local x-irradiation. Cancer 26, 232–238.

Suit, H.D., Zietman, A., Tomkinson, K., Ramsay, J., Gerweck, L., Sedlacek, R., 1990. Radiation response of xenografts of a human squamous cell carcinoma and a glioblastoma multiforme: a progress report. Int. J. Radiat. Oncol. Biol. Phys. 18, 365–373.

Sun, R., Champiat, S., Dercle, L., Aspeslagh, S., Castanon, E., Limkin, E.J., Baldini, C., Postel-Vinay, S., Hollebecque, A., Massard, C., Ammari, S., Deutsch, E., Soria, J.C., Marabelle, A., Ferté, C., 2017. Baseline lymphopenia should not be used as exclusion criteria in early clinical trials investigating immune checkpoint blockers (PD-1/PD-L1 inhibitors). Eur. J. Cancer 84, 202–211.

Sun, L., Clavijo, P.E., Robbins, Y., et al., 2019. Inhibiting myeloid-derived suppressor cell trafficking enhances T cell immunotherapy. JCI Insight 4, e126853.

Sun, G.Y., Wang, S.L., Song, Y.W., Jin, J., Wang, W.H., Liu, Y.P., Ren, H., Fang, H., Tang, Y., Zhao, X.R., Song, Y.C., Yu ZH, Liu X.F., Li, Y.X., 2020. Radiation-induced lymphopenia predicts poorer prognosis in patients with breast cancer: a post hoc analysis of a randomized controlled trial of postmastectomy hypofractionated radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. 108, 277–285.

Sundahl, N., Seremet, T., Van Dorpe, J., Neyns, B., Ferdinande, L., Meireson, A., Brochez, L., Kruse, V., Ost, P., 2019. Phase 2 trial of nivolumab combined with stereotactic body radiation therapy in patients with metastatic or locally advanced inoperable melanoma. Int. J. Radiat. Oncol. Biol. Phys. 104, 828–835.

Suzuki, R., Wei, X., Allen, P.K., Cox, J.D., Komaki, R., Lin, S.H., 2019. Prognostic significance of total lymphocyte count, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio in limited-stage small-cell lung cancer. Clin. Lung Cancer 20, 117–123.

Swietach, P., Vaughan-Jones, R.D., Harris, A.L., Hulikova, A., 2014. The chemistry, physiology and pathology of pH in cancer. Philos. Trans. R. Soc. Lond. B Biol. Sci. 369, 20130099.

Tang, C., Liao, Z., Gomez, D., Levy, L., Zhuang, Y., Gebremichael, Ra, Hong, Ds, Komaki, R., Welsh, Jw., 2014. Lymphopenia association with gross tumor volume and lung V5 and its effects on non-small cell lung cancer patient outcomes. Int. J. Radiat. Oncol. Biol. Phys. 89, 1084–1091.

Tarpley, J.L., Potvin, C., Chretien, P.B., 1975. Prolonged depression of cellular immunity in cured laryngopharyngeal cancer patients treated with radiation therapy. Cancer 35, 638–644.

Tredan, O., Menetrier-Caux, C., Ray-Coquard, I., Garin, G., Cropet, C., Verronese, E., et al., 2015. ELYPSE-7: a randomized placebo-controlled phase IIa trial with CYT107 exploring the restoration of CD4+ lymphocyte count in lymphopenic metastatic breast cancer patients. Ann. Oncol. 26, 1353–1362.

Tseng, Yd, Nguyen, Mh, Baker, K., Cook, M., Redman, M., Lachance, K., Bhatia, S., Liao, Jj, Apisarnthanarax, S., Nghiem, Pt, Parvathaneni, U., 2018. Effect of patient immune status on the efficacy of radiation therapy and recurrence-free survival among 805 patients with merkel cell carcinoma. Int. J. Radiat. Oncol. Biol. Phys. 102, 330–339.

Vaupel, P., Kallinowski, F., Okunieff, P., 1989. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res. 49, 6449–6465.

Venkatesulu, B.P., Mallick, S., Lin, S.H., Krishnan, S., 2018. A systematic review of the influence of radiation-induced lymphopenia on survival outcomes in solid tumors. Crit. Rev. Oncol. Hematol. 123, 42–51.

Vermorken, J.B., Claessen, A.M., van Tinteren, H., Gall, H.E., Ezinga, R., Meijer, S., Scheper, R.J., Meijer, C.J., Bloemena, E., Ransom, J.H., Hanna, M.G., Jr., Pinedo, H.M., 1999. Active specific immunotherapy for stage II and stage III human colon cancer: a randomized trial. Lancet. 353, 345–350.

Vigna-Pérez, M., Abud-Mendoza, C., Portillo-Salazar, H., et al., 2005. Immune effects of therapy with Adalimumab in patients with rheumatoid arthritis. Clin. Exp. Immunol. 141, 372–380.

Visage, R., Kaiser, N., Williams, M., Kim, A., 2019. Oral methods of urinary alkalinization for high-dose methotrexate administration: alternatives to intravenous sodium bicarbonate during a critical drug shortage. J. Pediatr. Hematol. Oncol. 41, 371–375.

Vögelin, M., Biedermann, L., Frei, P., et al., 2016. The impact of azathioprine-associated lymphopenia on the onset of opportunistic infections in patients with inflammatory bowel disease. PLoS One 11, e0155218.

Wang, L., Simons, D.L., Lu, X., Tu, T.Y., Solomon, S., Wang, R., Rosario, A., Avalos, C., Schmolze, D., Yim, J., Waisman, J., Lee, P.P., 2019. Connecting blood and intratumoral T(reg) cell activity in predicting future relapse in breast cancer. Nat. Immunol. 20, 1220–1230.

Weed, D.T., Vella, J.L., Reis, I.M., et al., 2015. Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. Clin. Cancer Res. 21, 39–48.

Wei, Y., Sticca, R.P., Holmes, L.M., Burgin, K.E., Li, J., Williamson, J., Evans, L., Smith, S.J., Stephenson, J.J., Wagner, T.E., 2006. Dendritoma vaccination combined with low dose interleukin-2 in metastatic melanoma patients induced immunological and clinical responses. Int. J. Oncol. 28, 585–593.

Weide, B., Martens, A., Hassel, J.C., Berking, C., Postow, M.A., Bisschop, K., Simeone, E., Mangana, J., Schilling, B., Di Giacomo, A.M., Brenner, N., Kähler, K., Heinzerling, L., Gutzmer, R., Bender, A., Gebhardt, C., Romano, E., Meier, F., Martus, P., Maio, M., Blank, C., Schadendorf, D., Dummer, R., Ascierto, P.A., Hospers, G., Garbe, C., Wolchok, J., 2016. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. Clin. Cancer Res. 22, 5487–5496.

Westermann, J., Reich, G., Kopp, J., Haus, U., Dörken, B., Pezzutto, A., 2001. Granulocyte/macrophage-colony-stimulating-factor plus interleukin-2 plus interferon alpha in the treatment of metastatic renal cell carcinoma: a pilot study. Cancer Immunol. Immunother. 49, 613–620.

Wigerup, C., Påhlman, S., Bexell, D., 2016. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol. Ther. 164, 152–169.

Wild, A.T., Herman, J.M., Dholakia, A.S., Moningi, S., Lu, Y., Rosati, L.M., Hacker-Prietz, A., Assadi, R.K., Saeed, A.M., Pawlik, T.M., Jaffee, E.M., Laheru, D.A., Tran, P.T., Weiss, M.J., Wolfgang, C.L., Ford, E., Grossman, S.A., Ye, X., Ellsworth, S.G., 2016. Lymphocyte-sparing effect of stereotactic body radiation therapy in patients with unresectable pancreatic cancer. Int. J. Radiat. Oncol. Biol. Phys. 94, 571–579.

Wozniak, K., Gloc, E., Morawiec, Z., Blasiak, J., 2008. Amifostine can differentially modulate DNA double-strand breaks and apoptosis induced by idarubicin in normal and cancer cells. Exp. Oncol. 30, 22–28.

Yamamoto, N., Nokihara, H., Yamada, Y., Shibata, T., Tamura, Y., Seki, Y., Honda, K., Tanabe, Y., Wakui, H., Tamura, T., 2017. Phase I study of Nivolumab, an anti-PD-1 antibody, in patients with malignant solid tumors. Invest. New Drugs 35, 207–216.

Yoshino, Y., Taguchi, A., Takao, M., Kashiyama, T., Furusawa, A., Uno, M., Okada, S., Kino, N., Yasugi, T., 2019. Lymphopenia after induction chemotherapy correlates with incomplete surgical resection in patients with advanced ovarian cancer. Int. J. Clin. Oncol. 24, 428–436.

Yuan, C., Wang, Q., 2018. Comparative analysis of the effect of different radiotherapy regimes on lymphocyte and its subpopulations in breast cancer patients. Clin. Transl. Oncol. 20, 1219–1225.

Zhang, H.G., Yang, P., Jiang, T., Zhang, J.Y., Jin, X.J., Hu, Y., Sun, J., Du, S.S., Zeng, Z.C., 2019. Lymphopenia is associated with gross target volumes and fractions in hepatocellular carcinoma patients treated with external beam radiation therapy and also indicates worse overall survival. Can. J. Gastroenterol. Hepatol. 2019, 9691067.

Zhao, Q., Chen, G., Ye, L., Shi, S., Du, S., Zeng, Z., He, J., 2019. Treatment-duration is related to changes in peripheral lymphocyte counts during definitive radiotherapy for unresectable stage III NSCLC. Radiat. Oncol. 14, 86.

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