REVIEW

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Trial watch
Chemotherapy with immunogenic cell death inducers

Erika Vacchelli,[1,2,3,†] Lorenzo Galluzzi,[1,2,3,†] Wolf Hervé Fridman,[2,4,5] Jerome Galon,[2,4,6] Catherine Sautès-Fridman,[2,4,7] Eric Tartour,[2,8,9] Laurence Zitvogel[2,10] and Guido Kroemer[1,2,8,11,*]

[1]U848; Villejuif, France; [2]INSERM; [3]Université Paris-Sud/Paris XI; Paris, France; [4]U872; Paris, France; [5]Pôle de Biologie; Hôpital Européen Georges Pompidou; AP-HP; Paris, France; [6]Université Pierre et Marie Curie/Paris VI; Paris, France; [7]Université Pierre et Marie Curie/Paris VI; Paris, France; [8]Pôle de Biologie; Hôpital Européen; Georges Pompidou; AP-HP; Paris, France; [9]Université Paris Descartes; Sorbonne Paris Cité; Paris, France; [10]Institut Gustave Roussy; Villejuif, France; [11]Université Paris Descartes; Sorbonne Paris Cité; Metabolomics Platform; Institut Gustave Roussy; Villejuif, France

[†]These authors contributed equally to this article.

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Abbreviations: CRT, calreticulin; DCs, dendritic cells; ER, endoplasmic reticulum; GM-CF, granulocyte-macrophage colony-stimulating factor; HMGB1, high mobility group box 1; HSPs, heat-shock proteins, ICD, immunogenic cell death; IFNγ, interferon γ; IL, interleukin; NSCLC, non-small cell lung cancer; TACE, transhepatic artery chemoembolization; TLR4, Toll-like receptor 4; Tregs, FOXP3[+] regulatory T cells

The long-established notion that apoptosis would be immunologically silent, and hence it would go unnoticed by the immune system, if not tolerogenic, and hence it would actively suppress immune responses, has recently been revisited. In some instances, indeed, cancer cells undergo apoptosis while emitting a spatiotemporally-defined combination of signals that renders them capable of eliciting a long-term protective antitumor immune response. Importantly, only a few anticancer agents can stimulate such an immunogenic cell death. These include cyclophosphamide, doxorubicin and oxaliplatin, which are currently approved by FDA for the treatment of multiple hematologic and solid malignancies, as well as mitoxantrone, which is being used in cancer therapy and against multiple sclerosis. In this Trial Watch, we will review and discuss the progress of recent (initiated after January 2008) clinical trials evaluating the off-label use of cyclophosphamide, doxorubicin, oxaliplatin and mitoxantrone.

Introduction

For a long time, cell death instances were cataloged into either of two mutually exclusive and diametrically opposed categories: necrosis, constituting an accidental, pathological, morphologically nebulous and pro-inflammatory cell death subroutine, and apoptosis, being a finely regulated, physiological, morphologically defined and immunologically silent (if not tolerogenic) one.[1-3] During the last decade, this textbook dichotomy has been revisited, if not invalidated. Thus, multiple pieces of evidence have accumulated to disconfirm the near-to-dogmatic notions that necrosis would constitute a merely accidental cell death mode,[4] that necrosis would fail to exhibit peculiar morphological manifestations,[5] that necrosis would not be involved in physiological processes,[5] and that apoptosis would always fail to elicit inflammatory and immune responses.[6]

In 2005, Casares et al. first reported that tumor cells succumbing in vitro to anthracyclines, notably doxorubicin (but not to other chemotherapeutics such as mitomycin C), can acquire the capacity to elicit tumor-specific immune responses when inoculated in syngenic mice.[7] Such immune responses were found to efficiently protect mice against subsequent re-challenges with live cells of the same type, de facto resulting in long-term vaccination.[7] Since then, great efforts have been dedicated to the elucidation of the molecular and cellular mechanisms that underlie immunogenic cell death (ICD), leading to the discovery that ICD relies on the emission of a spatiotemporally-defined combination of signals by dying cells.[8] Such signals include (though perhaps are not limited to),[9] (1) the endoplasmic reticulum (ER) stress-elicited, caspase-dependent pre-apoptotic co-exposure of the ER chaperons calreticulin (CRT) and ERp57 on the outer leaflet of the plasma membrane;[10-14] (2) the autophagy-dependent pre-apoptotic secretion of ATP;[15-17] (3) the post-apoptotic release of the non-histone chromatin binding protein high mobility group box 1 (HMGB1)[18]; and (4) the cell surface exposure or release of heat-shock proteins (HSPs) including HSP70 and HSP90.[19,20] For ICD to be productive, i.e., to elicit a long-term protective anticancer immune response, each of these signals must be properly decoded by the immune system. Thus, by binding to a hitherto uncharacterized receptor on the surface of dendritic cells (DCs), CRT acts as an "eat-me" signal, thereby stimulating the DC-mediated uptake of apoptotic corpses (and hence tumor antigens).[10,21] Extracellular ATP not only functions as a "find-me" signal, thereby stimulating the local recruitment of immune effector cells,[22] but also binds to purinergic P2RX7 receptors on the surface of these cells, thereby triggering the activation of the NLRP3 inflammasome.[17,23] This is a critical step for the induction

*Correspondence to: Guido Kroemer; Email: kroemer@igr.fr
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of antitumor immunity, as the inflammasome catalyzes the proteolytic maturation and secretion of interleukin-1β (IL-1β), a cytokine that is required for the adequate polarization of interferon γ (IFNγ)-producing $CD8^+$ T cells.[17] By binding to Toll-like receptor 4 (TLR4) on DCs, HMGB1 engages a MYD88-mediated signaling cascade leading to increased tumor antigen processing and cross-presentation to T cells.[24] Along similar lines, the presence of HSPs on the surface of dying tumor cells or in their vicinity promotes the formation of tumor antigen-HSP complexes that are processed by DCs for T cell cross-priming more efficiently than tumor antigens alone.[25]

So far, only a few anticancer agents have been shown to kill tumor cells while inducing all these phenomena in the correct spatiotemporal order, thus eliciting bona fide ICD. These include some types of radiotherapy and only four chemotherapeutics: the DNA alkylating compound cyclophosphamide, the anthracyclines doxorubicin and mitoxantrone and the platinum derivative oxaliplatin.[7,10,26-28] Importantly, anticancer agents failing to elicit one (or more) of the abovementioned sine quibus non are intrinsically unable to induce ICD, a defect that, at least in some instances, can be restored by targeted pharmacological interventions. Thus, cisplatin (a platinum derivative structurally related to oxaliplatin) alone fails to elicit the pre-apoptotic exposure of CRT, yet becomes able to do so when combined with ER stressors such as thapsigargin, rendering cisplatin-induced cell death immunogenic.[29] Recently, the histone deacetylase inhibitor vorinostat has been found to trigger CRT exposure in childhood brain tumor cell lines, in vitro.[30] However, the true potential of vorinostat as an inducer of ICD remain unexplored, and actually contrasting reports can be found in the literature on its immunostimulatory vs. immunosuppressive effects.[31-33]

At present, cyclophosphamide, doxorubicin and oxaliplatin are approved by FDA for the treatment of multiple malignancies (**Table 1**). Mitoxantrone is mainly used for a cancer-unrelated indication, multiple sclerosis, even though FDA has also approved mitoxantrone-containing combination regimens for the treatment of acute leukemia, non-Hodgkin's lymphoma, breast and prostate cancer (**Table 1**). In this Trial Watch, we will discuss the progress of recent (started after January 2008) clinical studies evaluating the efficacy—as off-label medications—of the only four chemotherapeutics that so far have been described as bona fide ICD inducers.

Cyclophosphamide

Cyclophosphamide is a DNA alkylating agent belonging to the family of nitrogen mustards.[34] Upon conversion into 4-hydroxycyclophosphamide by hepatic mixed function oxidases, cyclophosphamide becomes able to add an alkyl group (C_nH_{2n+1}) to the nitrogen atom at position 7 in the imidazole group of purine DNA bases, thereby acquiring cytotoxic properties.[34] Although initially conceived as a cancer-selective drug (due to a presumed cancer cell-specific mechanism of activation), cyclophosphamide has rapidly turned out to be cytotoxic for multiple cell types, including immune cells.[34] Owing to this pharmacodynamic profile, cyclophosphamide has soon entered the clinical practice not only as an anticancer agent for the treatment of some forms of lymphoma, leukemia and solid tumors (**Table 1**), but also for the therapy of non-neoplastic autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis.[35] Still, recent data indicate that, at odds with the immunosuppressive properties of cyclophosphamide at high doses, metronomic cyclophosphamide regimens exert profound immunostimulatory effects,[36] for instance by selectively depleting or inhibiting $FOXP3^+$ regulatory T cells (Tregs).[37] Such immunostimulatory properties appear to at least contribute to, if not entirely explain, the therapeutic success of cyclophosphamide as an anticancer agent.[34]

In the last three years (2009–2011), a consistent number of trials aimed at testing the clinical benefits of cyclophosphamide in cancer patients has been terminated and the corresponding results published in high impact scientific journals (source http://www.ncbi.nlm.nih.gov/sites/entrez). In most cases, these clinical studies investigated—in oncological settings in which cyclophosphamide is approved by FDA—either how variations in dosage and/or schedule affect safety and efficacy or whether the combination between cyclophosphamide and other chemotherapeutics improves the therapeutic outcome.[38-42] In addition, cyclophosphamide-based combination regimens have been tested in both low- and intermediate-risk rhabdomyosarcoma pediatric patients, in both cases providing no significant benefits as compared with the control arm.[43,44]

At present, there are around 530 open clinical trials, all phases confounded, that test the efficacy of cyclophosphamide in oncological indications (source www.clinicaltrials.gov). Approximately

Table 1. Currently approved indications for immunogenic chemotherapy*

Drug	Indications
Cyclophosphamide	ALL, AML, breast cancer, CLL, CML, lupus nephritis, lymphoma, multiple myeloma, mycosis fungoides, neuroblastoma, nephrotic syndrome, ovarian cancer, retinoblastoma.
Doxorubicin	ALL, AML, breast cancer, bronchogenic carcinoma, cervical carcinoma, gastric carcinoma, germ cell tumors, hepatic carcinoma, HNC, lymphoma, mesothelioma, multiple myeloma, neuroblastoma, ovarian carcinoma, pancreatic carcinoma, prostate cancer, SCLC, soft tissue and bone sarcomas, thyroid carcinoma, transitional cell bladder carcinoma, uterine carcinoma, Wilms' tumor.
Oxaliplatin	Metastatic colorectal cancer, ovarian cancer.
Mitoxantrone	Acute leukemia, breast cancer, NHL, multiple sclerosis, prostate cancer.

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HNC, head and neck cancer; NHL, non-Hodgkin's lymphoma; SCLC, small cell lung cancer. *by FDA or European Medicines Agency (EMA) at the day of submission.

430 (320–355 phase I-II, 75–110 phase III-IV) of these studies are performed in clinical scenarios that correspond or overlap with cyclophosphamide indications. In addition, cyclophosphamide is being evaluated, alone or in combination with other anticancer agents, in several off-label settings (**Table 2**). Around 10% of these latter trials are advanced ones (phase III-IV), including four studies that test cyclophosphamide-including combination regimens in brain cancer—notably atypical teratoid/rhabdoid tumors, choroid plexus tumors and ependymoma—patients (NCT01014767, NCT01096368, NCT00683319, NCT00653068), as well as two studies that investigate the combination of cyclophosphamide with cancer vaccines in non-small cell lung cancer (NSCLC) patients (NCT01015443, NCT01444118). A consistent fraction of early trials (phase I-II) is testing whether cyclophosphamide (most often in association with the nucleoside analog fludarabine or with immunostimulatory interventions such as the administration of

Table 2. Main trends of clinical trials evaluating the effects of cyclophosphamide as an off-label medication for cancer patients*

Location	Tumor type	Trials*	Phase	Notes
Early clinical trials (phase I-II)				
Brain	Choroid plexus carcinoma	1	II	Often combined with platinum-containing regimens, EGFR inhibitors, etoposide or peptide-based vaccines.
	Embryonic brain tumors	1	II	
	Ependymoma	1	II	
	Glioblastoma	2	I-II	
	Medulloblastoma	2	II	
Colorectal tract	CRC	5	I-II	Combined with different immunostimulatory approaches.
Connective tissue	Osteosarcoma	1	II	Combined with sirolimus.
	Rhabdomyosarcoma	2	II	Combined with mAbs.
Epidermis	Melanoma	20	I-II	Often combined with fludarabine or immunostimulatory interventions.
Gastrointestinal system	Pancreatic cancer	5	II	Often combined with GM-CSF-based vaccines.
Hematological tumors	ATL	1	II	Combined with fludarabine.
	MDS	3	II	Often combined with ATG, fludarabine and stem cell transplantation.
	T-PLL	1	II	Combined with fludarabine and immunotherapy.
HNC	SCCHN	2	I	Combined with fludarabine and/or immunostimulatory interventions.
Kidney	Advanced or metastatic renal cancer	2	I-II	Combined with allogeneic HSCT, immunostimulatory interventions or everolimus.
Lung	Metastatic lung cancer	1	II	Combined with cancer vaccines.
	NSCLC	3	I-II	Combined with cancer vaccines.
	PPB	1	II	Combined with dactinomycin, doxorubicin, ifosfamide and vincristine.
	SCLC	1	II	In the context of the PCDE regimen.
Mesothelioma	-	2	I	Combined with immunotherapy.
Reproductive tract	Prostate cancer	5	I-II	Often combined with immunostimulatory approaches.
Thymus	Thymoma	2	I-II	Combined with belinostat, cetuximab, cisplatin or doxorubicin.
Advanced clinical trials(phase III-IV)				
Brain	AT/RT	1	III	Combined with cisplatin, etoposide, folinic acid, methotrexate and vincristine.
	Choroid plexus tumors	1	III	Combined with platinum-containing anticancer drugs, etoposide and vincristine.
	Ependymoma	2	III	Always combined with platinum-containing anticancer drugs and vincristine.
Lung	NSCLC	2	III	Always in combination with cancer vaccines.

Abbreviations: ATG, thymoglobulin; ATL, adult T-cell leukemia; AT/RT, atypical teratoid/rhabdoid tumor; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HNC, head and neck cancer; HSCT, hematopoietic stem cell transplantation; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; NSCLC, non-small cell lung cancer; PCDE, cisplatin, cyclophosphamide, doxorubicin, etoposide; PPB, pleuropulmonary blastoma; SCCHN, squamous cell cancer of the head and neck; SCLC, small cell lung carcinoma; T-PLL, T-cell prolymphocytic leukemia. *started after January, 1st 2008 and not completed or terminated at the day of submission.

IL-2, DC-based vaccines or autologous T cell transfers) is safe and efficient in melanoma patients (NCT01005745, NCT00863330, NCT01339663, NCT01106235, NCT01435499, NCT00683670, NCT00937625, NCT00871481, NCT00722098, NCT01455259, NCT00604136, NCT00846833, NCT00910650, NCT01319565, NCT01236573, NCT01468818, NCT01369875, NCT01369888, NCT01271907, NCT01118091). The combination between cyclophosphamide and granulocyte-macrophage colony-stimulating factor (GM-CSF)-based vaccines (including GVAX) in the context of pancreatic cancer is being investigated by many groups (NCT01468870, NCT01417000, NCT01088789, NCT00727441, NCT01021800). Along similar lines, cyclophosphamide is currently being tested together with immunostimulatory approaches (including anti-PD1 and anti-OX40 monoclonal antibodies)[45] against colorectal (NCT01064375, NCT00785122, NCT01462513, NCT00986518) and prostate cancer (NCT01303705, NCT01093183, NCT01420965, NCT01140373, NCT00753220). Besides these main trends, there are several other oncological indications for which the putative benefits of cyclophosphamide are being investigated in phase I-II clinical trials (**Table 2**), including (but not limited to) glioblastoma (NCT01403285, NCT01454596), myelodysplastic syndrome (NCT01255319, NCT00604201, NCT01174108), medulloblastoma (NCT00867178, NCT01356290), NSCLC (NCT00960115, NCT01159288), renal cancer (NCT01462214, NCT00923845), rhabdomyosarcoma (NCT01222715, NCT01055314) and thymoma (NCT01100944, NCT01025089).

Taken together, these observations clearly suggest that cyclophosphamide is the subject of an intense wave of clinical investigation, most often owing to its multifaceted immunostimulatory functions. Importantly, while many clinical studies demonstrate that metronomic cyclophosphamide leads to improved T cell effector functions,[36,46-48] its long-term clinical efficacy as well as its added value as compared with other strategies for depleting/inhibiting Tregs remain a matter of debate.[49-52] The results from ongoing clinical trials will help to clarify this issue.

Doxorubicin

Doxorubicin (also known as adriamycin) is a natural compound belonging to the class of anthracycline antibiotics that functions as a DNA intercalating agent.[53] As such, it impedes the progression of topoisomerase II along DNA, de facto blocking the resolution of quaternary DNA structures (while inducing an accumulation of topoisomerase-introduced single-strand breaks) and hence inhibiting DNA replication (and to a lesser degree transcription).[54] Doxorubicin is associated with common and relatively mild side effects, including nausea, vomiting and alopecia, as well as with dose-limiting cardiotoxicity, presumably due to the generation of mitochondriotoxic reactive oxygen species (ROS) upon the interaction between doxorubicin and iron.[55] Still, doxorubicin (alone or in combination with other chemotherapeutics including cyclophosphamide) is widely used for the treatment of some forms of leukemia, Hodgkin's lymphoma as well as a plethora of solid neoplasms, including (but not limited to) bladder, breast, stomach, lung, ovarian and thyroid cancer (**Table 1**). Recent preclinical results from several laboratories worldwide indicate that doxorubicin not only triggers ICD but stimulates various aspects of the immune response against cancer.[36]

During the last triennium, only a few doxorubicin-based clinical trials have been reported in major scientific journals (source www.clinicaltrials.gov), focusing on the optimization of dosage and schedule for FDA-approved indications.[39,40] Now, approximately 370 open clinical trials, all phases confounded, are investigating the efficacy of doxorubicin against various types of cancer (source www.clinicaltrials.gov). Among these, some 270 trials (190–220 phase I-II, 50–80 phase III-IV) are performed in settings that match or exhibit some degree of overlaps with the FDA-approved indications of doxorubicin. Moreover, doxorubicin is being tested, alone or combined with other chemotherapeutics, as an off-label medication in multiple clinical scenarios (**Table 3**). Indicatively, 20% of these latter trials are advanced ones, including a large fraction of studies in which doxorubicin is tested for the neoadjuvant treatment of liver cancer, either in combination with chemotherapy or coupled to maneuvers for maximizing efficacy such as transhepatic artery chemoembolization (TACE) (NCT01387932, NCT01332669, NCT00617981, NCT00936689, NCT01327521, NCT00807300, NCT01015833, NCT01324076, NCT01004978, NCT00980460). Other advanced trials explore the use of doxorubicin against lymphoma (NCT00854568, NCT00722137), multiple myeloma (NCT00734877, NCT00670631) and endometrial cancer (NCT00883116, NCT00698620). Along similar lines, a consistent fraction of doxorubicin-based early clinical trials (**Table 3**) is performed on patients affected by liver cancer (often in association with TACE or the anti-angiogenic compound sorafenib) (NCT00988195, NCT01125020, NCT00844883, NCT00877071, NCT01259024, NCT01033578, NCT01116635, NCT01272557, NCT00855218, NCT00990860, NCT00919009, NCT01381211, NCT00857805, NCT01009801, NCT01281943, NCT01011010, NCT00949182, NCT00956930), multiple myeloma (near-to-always in combination with the proteasomal inhibitor bortezomib) (NCT00720174, NCT00750815, NCT01215344, NCT00617591, NCT01246063, NCT00985907, NCT00863174, NCT00849251, NCT01394354, NCT00706953, NCT01177683, NCT00724568, NCT00872521, NCT01160484, NCT01101594, NCT01365559, NCT00925821, NCT01255514. NCT01371227, NCT00744354, NCT00814541, NCT01078441. NCT00742404, NCT01055301, NCT01328236) and endometrial cancer (NCT01100359, NCT00739830). In addition, the safety and efficacy of doxorubicin (in most cases combined with other chemotherapeutics) are being investigated in diffuse large B-cell lymphoma (NCT01361191, NCT01087424), osteosarcoma (NCT01459484, NCT00691236, NCT01258634), thymoma (NCT01025089, NCT01100944) and urothelial cancer patients (NCT01093066, NCT00808639).

In spite of the fact that both cyclophosphamide and doxorubicin have been recently ascribed with a consistent immunostimulatory potential, in particular when employed in metronomic regimens,[36] only for the former this notion appears to translate into a clinical interest. The reasons underlying this trend remain unclear, yet may be related to toxicological and/or economical factors.

Oxaliplatin

Oxaliplatin is a third-generation platinum coordination complex originally developed in the 1990s in the attempt to circumvent the common resistance of tumors against first- (cisplatin) and second-generation (carboplatin) compounds.[56] The molecular mechanisms whereby platinum derivatives, especially oxaliplatin, exert cytotoxic effects against cancer cells are complex and surely overtake their capacity to generate DNA adducts and to activate the pro-apoptotic transcription factor p53.[57-60] Although cisplatin-refractory neoplasms are largely considered to be responsive to oxaliplatin, clinical data suggest that there may be some degree of cross-resistance.[61] Since 1996 in Europe and 2002 in the US, oxaliplatin is clinically employed for the treatment of colorectal cancer in combination with 5-fluorouracil and folinic acid (the FOLFOX protocol) (**Table 1**). Besides inducing ICD, oxaliplatin reportedly inhibits the expression of programmed death ligand 2 (PD-L2), thereby limiting immunosuppression by both DCs and tumor cells.[62]

Table 3. Main trends of clinical trials evaluating the effects of doxorubicin as an off-label medication for cancer patients*

Location	Tumor type	Trials*	Phase	Notes
Early clinical trials (phase I-II)				
Connective tissue	MFH	2	I/II	Combined with mAb and chemotherapeutics.
	Osteosarcoma	4	II	Always combined with cisplatin.
	Rhabdomyosarcoma	1	II	Combined with mAb and chemotherapeutics.
Hematological tumors	DLBCL	2	II	Within the R-MEGACHOP or R-MiniCHOP regimen.
	Leukemia	1	II	Combined with bortezomib and dexamethasone.
	Plasma cell neoplasms (including MM)	23	I-II	Often combined with bortezomib, cyclophosphamide and dexamethasone.
Epidermis	Melanoma	2	I-II	In the context of TACE.
Liver	HCC	14	I-II	Often combined with sorafenib and TACE.
	Non-specified	4	I-II	Often combined with mitomycin C and sorafenib.
Lung	NSCLC	1	II	Combined with carboplatin.
	Pleural mesothelioma	2	II	Always combined with cisplatin.
	PPB	1	II	Combined with cyclophosphamide, dactinomycin, ifosfamide and vincristine.
Reproductive tract	Endometrial cancer	1	II	Often combined with hormonotherapy.
	Prostate cancer	2	II	
Thymus	Thymoma	2	I-II	Combined with belinostat, cetuximab, cisplatin and cyclophosphamide.
Urogenital tract	Bladder cancer	1	II	In the context of the MVAC regimen.
	Urothelial tract	3	II	
Advanced clinical trials(phase III-IV)				
Brain	Choroid plexus tumors	1	III	Always combined with cyclophosphamide and etoposide.
	Others	1	III	
Hematological tumors	Lymphomas	2	III	Within the CHOP or R-CHOP regimen.
	MM	2	III	Within the M-VTD-PACE or DPACE regimen.
Liver	HCC	6	III-IV	In the context of TACE.
	Others	4	III	Combined with other chemotherapeutics.
Reproductive tract	Endometrial cancer	2	III	Alone or combined with cisplatin, filgrastim or paclitaxel.

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; DLBCL, diffuse large B-cell lymphoma; DPACE, dexamethasone, cisplatin, doxorubicin, cyclophosphamide, etoposide; HCC, hepatocellular carcinoma; mAb, monoclonal antibody; M-VTD-PACE, bortezomib, cisplatin, cyclophosphamide, dexamethasone, etoposide, melphalan, thalidomide; MFH, malignant fibrous histiocytoma; MM, multiple myeloma; MVAC, doxorubicin, cisplatin, methotrexate, vinblastine; NSCLC, non-small cell lung cancer; PPB, pleuropulmonary blastoma; R-CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab; R-MEGACHOP, cyclophosphamide, etoposide, ifosfamide, prednisone, rituximab, vincristine; R-MiniCHOP, cyclophosphamide, prednisone, rituximab, vincristine; TACE, transhepatic arterial chemoembolization. *started after January, 1st 2008 and not completed or terminated at the day of submission.

The triennium 2009–2011 has witnessed several high impact publications on the clinical profile of oxaliplatin (source www.clinicaltrials.gov). On one hand, several studies have evaluated dosage, schedule and the possible combination of oxaliplatin with other chemotherapeutics for the therapy of colorectal cancer.[63-65] On the other hand, multiple reports suggest that oxaliplatin may constitute a valuable therapeutic option for a wide range of tumors, including NSCLC, pancreatic, biliary tract, gall bladder and ampullary cancer.[66-72]

Not less than 330 ongoing clinical trials, all phases confounded, currently investigate the efficacy of oxaliplatin in cancer patients (source www.clinicaltrials.gov). Approximately 190 (140–160 phase I-II, 30–50 phase III-IV) of these studies involve colorectal cancer patients. In addition, there are some 140 clinical trials that evaluate the potential of oxaliplatin, alone or combined with other interventions, as an off-label medication (**Table 4**). Among 12 advanced clinical trials, three investigate the efficacy of oxaliplatin plus gemcitabine (a nucleoside analog) in biliary tract cancer patients (NCT01313377, NCT01470443, NCT01149122). In addition, there are four phase III trials evaluating the benefits of oxaliplatin (combined with the nucleoside analog 5-fluorouracil or with its pro-drug capecitabine) against gastric cancer (NCT01470742, NCT00941655, NCT00718354, NCT00680901), and three phase III trials in which oxaliplatin (associated to nucleoside analogs) is tested in pancreatic cancer patients (NCT01314027, NCT01362582, NCT01121848). This trend is fully reflected in early clinical studies, including 10 trials on biliary tract cancer patients (NCT01267344, NCT01180153, NCT01234051, NCT01127555, NCT00881504, NCT01389414, NCT01247337, NCT00713687, NCT00779454, NCT01206049), 50 studies on esophageal and gastric cancer patients (NCT00711243, NCT01307956, NCT01191697, NCT00982592, NCT01246960, NCT01386346, NCT01443065, NCT00849615, NCT01333033, NCT01295086, NCT01362127, NCT01262482, NCT00673673, NCT00667420, NCT00816543, NCT00861094, NCT01467921, NCT01248299, NCT00711412, NCT00985556, NCT00961077, NCT01471132, NCT01364493, NCT01138904, NCT00952003, NCT01359397, NCT01396707, NCT01049620, NCT01216644, NCT01090505, NCT01331928, NCT01130805, NCT01129310, NCT00767377, NCT01364376, NCT00733616, NCT01283204, NCT01130337, NCT01070290, NCT01106066, NCT01160419, NCT01100801, NCT01197118, NCT01351038, NCT01472029, NCT01206218, NCT00854854, NCT01208103, NCT01202409, NCT01422993). In addition, oxaliplatin is being investigated (most often in combination with 5-fluorouracil) in 14 distinct clinical trials enrolling pancreatic cancer patients (NCT00690300, NCT01473303, NCT00707278, NCT01413022, NCT01397019, NCT01359007, NCT00786058, NCT01446458, NCT01063192, NCT00728000, NCT01394120, NCT01415713, NCT01209962, NCT01454180) as well as in eight studies based on prostate cancer patients (NCT01338792, NCT00871169, NCT01042028, NCT00636883, NCT00602602, NCT01048320, NCT00609336, NCT01383538).

Thus, there appears to be a consistent interest in the clinical properties of oxaliplatin, in particular in the context of combination regimens that include nucleoside analogs. Results from recently terminated clinical trials suggest that oxaliplatin may be beneficial for a wide range of solid tumors. Although it is too early to discern whether this depends or not on the ability of

Table 4. Main trends of clinical trials evaluating the effects of oxaliplatin as an off-label medication for cancer patients*

Location	Tumor type	Trials*	Phase	Notes
Early clinical trials (phase I-II)				
Breast	Breast cancer	5	II	In association with nucleoside analogs, docetaxel or trastuzumab.
Gastrointestinal system	Biliary tract cancer	9	II	Often combined with nucleoside analogs, including 5-FU, capecitabine or gemcitabine.
	Esophageal cancer	19	I-II	
	Gastric cancer	42	I-II	
	Pancreatic cancer	14	I-II	
Hematological tumors	Lymphomas	6	I-II	Combined with nucleoside analogs and/or glucocorticoids.
Liver	HCC	6	I-II	Often combined with nucleoside analogs.
Lung	NSCLC	3	II	Combined with docetaxel or pemetrexed.
Reproductive tract	Prostate cancer	8	I-II	Combined with nucleoside analogs or EGFR inhibitors.
Advanced clinical trials(phase III-IV)				
Gastrointestinal system	Biliary tract cancer	3	III	Always combined with gemcitabine.
	Gastric cancer	4	III	Combined with 5-FU or capecitabine.
	Pancreatic cancer	3	III	Combined with 5-FU or gemcitabine.

Abbreviations: 5-FU, 5-fluorouracil; EGFR, epidermal growth factor receptor; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer. *started after January, 1st 2008 and not completed or terminated at the day of submission.

oxaliplatin to trigger ICD, the oncological indications for which oxaliplatin is approved by FDA may soon increase.

Mitoxantrone

Similar to doxorubicin, mitoxantrone (a synthetic anthracenedione first developed in the mid 1980s) operates as an intercalating agent and inhibits topoisomerase II, thus impairing DNA replication, transcription and repair.[73] Mitoxantrone shares doxorubicin's spectrum of adverse reactions, including immunosuppression and a dose-limiting cardiotoxicity that can develop during treatment as well as years after discontinuation.[74] Due to its immunosuppressive properties, mitoxantrone is successfully used in the clinic to limit the frequency of relapse and slow the progression of several variants of multiple sclerosis.[75] In addition, the FDA has approved mitoxantrone, alone or in combination with other chemotherapeutics or prednisone, for the therapy of acute leukemia, non-Hodgkin's lymphoma, breast and prostate cancer (**Table 1**).

Results from recently terminated (2009–2011), randomized clinical trials confirm that mitoxantrone provides clinical benefits in children with acute lymphoblastic leukemia but suggest that cabazitaxel plus prednisone may be superior than mitoxantrone plus prednisone for the treatment of metastatic castration-resistant prostate cancer that progresses upon docetaxel-based therapy.[76,77]

Now, 36 open clinical trials are studying the efficacy of mitoxantrone against distinct types of cancer (source www.clinicaltrials.gov). Of these, 29 (23 phase I-II, 6 phase III-IV) are performed in settings that match mitoxantrone FDA-approved indications. Moreover, the safety and efficacy of mitoxantrone, alone or combined with other anticancer agents, are being evaluated in a few off-label scenarios (**Table 5**). Among these latter studies, one single advanced (phase III) trial is testing the association between mitoxantrone (or other chemotherapeutics) and the anti-CD20 monoclonal antibody rituximab in follicular lymphoma patients (NCT00774826). Along similar lines, mitoxantrone is currently being investigated in five early clinical trials for its efficacy against various types of lymphoma and T-cell prolymphocytic leukemia (NCT00901927, NCT01133158, NCT01186640, NCT01144403, NCT00712582).

The reasons whereby mitoxantrone—at odds with doxorubicin, which is also an anthracycline—is not the subject of an intense wave of clinical studies as an off label medication remain unclear. Perhaps, this may be due to the fact that while doxorubicin-based metronomic regimens have already been developed (and shown not only to be devoid of immunosuppressive effects but also to actively stimulate immunity),[36] the same does not hold true for mitoxantrone, whose preferential toxicity for immune cells de facto underlies its anticancer potential against lymphoma and leukemia.

Concluding Remarks

For a long time, the immune system has been viewed as a rather passive bystander of cancer, so much that even the National Cancer Institute recommended testing the antineoplastic potential of new molecules in immunodeficient murine models. Now, it has become clear that the immune system plays a critical role not only during early oncogenesis, by keeping under surveillance transformed and potentially tumorigenic cells, but also during the response of established malignancies to therapy.[36,78-80] On one hand, indicators of an ongoing immune response, such as the extent or the composition of the intratumoral infiltrate, as well as polymorphisms in genes that code for immune modulators have been correlated with the outcome of therapy. On the other hand, several anticancer compounds (be they conventional chemotherapeutics or targeted agents) have recently been shown to stimulate antitumor immunity.[36]

Among several molecular and cellular circuitries whereby anticancer agents can trigger tumor-specific immune responses stands the induction of ICD, a functionally peculiar type of apoptosis that is associated with a spatiotemporally defined combination of immunogenic signals.[8] In spite of the fact that the existence of ICD has been acknowledged only a few years ago,[10] some agents that are capable of triggering ICD have already been identified, including cyclophosphamide, doxorubicin, oxaliplatin and mitoxantrone. All these compounds are approved by FDA for cancer therapy, have been successfully used in the clinic for several years, and are now being investigated for their utility in a range of off-label applications. It is tempting to speculate—but cannot be formally demonstrated—that part of the clinical success of these chemicals is due to their ability to trigger ICD. Irrespective of these considerations, it will be interesting to see whether cyclophosphamide, doxorubicin, oxaliplatin and mitoxantrone will be approved for additional cancer-related indications, as well as if novel inducers of ICD will be identified and will make their way from the bench to the bedside. In addition, it remains to be seen whether ICD inducers may be advantageously combined with non-immunogenic conventional chemotherapeutics, targeted anticancer agents and/or immunostimulatory strategies.

Table 5. Main trends of clinical trials evaluating the efficacy of mitoxantrone as an off-label medication for cancer patients*

Location	Tumor type	Trials*	Phase	Notes
Early clinical trials (phase I-II)				
Hematological tumors	Lymphoma	4	II	Always in association with rituximab.
	T-PLL	1	II	In association with alemtuzumab, cyclophosphamide and fludarabine.
Advanced clinical trials(phase III-IV)				
Hematological tumors	Follicular lymphoma	1	III	In association with rituximab and fludarabine.

Abbreviations: T-PLL, T-cell prolymphocytic leukemia. *n° of trials started after January, 1st 2008 and not completed or terminated at the day of submission.

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