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Trial watch

Chemotherapy with immunogenic cell death inducers

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Abbreviations: CAR, chimeric antigen receptor; CDK, cyclin-dependent kinase; CRT, calreticulin; CTLA4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; DOS, docetaxel + oxaliplatin; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; FOLFIRINOX, folinic acid + 5-fluorouracil + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-fluorouracil + oxaliplatin; FRα, folate receptor α; G-CSF, granulocyte colony-stimulating factor; GEMOX, gemcitabine + oxaliplatin; GM-CSF, granulocyte-macrophage colony-stimulating factor; HIFU, high intensity focused ultrasound; HMGB1, high mobility group box 1; HSP, heat-shock protein; ICD, immunogenic cell death; IL, interleukin; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung carcinoma; PARP, poly(ADP-ribose) polymerase 1; PLD, pegylated liposomal doxorubicin; ROS, reactive oxygen species; SOX, S-1 + oxaliplatin; TLR, Toll-like receptor; VEGF, vascular endothelial growth factor; XELOX, capecitabine + oxaliplatin

It is now clear that the immune system plays a critical role not only during oncogenesis and tumor progression, but also as established neoplastic lesions respond to therapy. Selected cytotoxic chemicals can indeed elicit immunogenic cell death, a functionally peculiar type of apoptosis that stimulates tumor-specific cognate immune responses. Such immunogenic chemotherapeutics include cyclophosphamide, doxorubicin and oxaliplatin (which are approved by FDA for the treatment of various hematological and solid malignancies), mitoxantrone (which is currently employed both as an anticancer agent and against multiple sclerosis) and patupilone (a microtubular poison in clinical development). One year ago, in the second issue of *OncoImmunology*, we discussed the scientific rationale behind immunogenic chemotherapy and reviewed the status of recent clinical trials investigating the off-label use of cyclophosphamide, doxorubicin, oxaliplatin and mitoxantrone in cancer patients. Here, we summarize the latest developments in this area of clinical research, covering both high-impact studies that have been published during the last 13 months and clinical trials that have been initiated in the same period to assess the antineoplastic profile of immunogenic chemotherapeutics.

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Introduction

The long-standing notion that apoptosis would invariably constitute an immunologically silent (and hence would fail to activate the immune system), if not a tolerogenic (and hence would actively suppress immune responses), cell death modality has recently been invalidated.[1,2] Thus, at least in selected circumstances, cancer cells die while emitting a spatiotemporally defined combination of signals that results in the activation of tumor-specific cognate immune responses.[3,4] The signals that have been associated with the productive perception of immunogenic cell death (ICD) by the immune system include, though presumably are not limited to, (1) the pre-apoptotic exposure of the endoplasmic reticulum (ER) chaperone calreticulin (CRT) and heat-shock proteins (HSPs), including HSP70 and HSP90, on the cell surface,[5–8] (2) the autophagy-dependent active secretion of ATP[9,10] and (3) the post-apoptotic release of the nuclear non-histone chromatin-binding protein high mobility group box 1 (HMGB1).[11] By binding to CD91 on the surface of dendritic cells (DCs), CRT functions as an "eat-me" signal, thus promoting the engulfment of apoptotic corpses.[5,12] The presence of HSPs on the surface or in the proximity of dying cancer cells results in the formation of tumor antigen-HSP complexes, which are processed by DCs for the cross-priming of T cells more efficiently than tumor antigens alone.[13] Extracellular ATP stimulates the NLRP3 inflammasome, a platform for the activation of caspase-1,[14] via purinergic P2RX7 receptors, hence promoting the proteolytic maturation and the release of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-18.[15] Finally, HMGB1 exerts

Table 1. Approved indications for immunogenic chemotherapy*,**

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

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; 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. **updated from ref. [44].

immunogenic functions via a Toll-like receptor 4 (TLR4), and MYD88-dependent signaling cascade that boosts the processing and cross-presentation of antigens from dying tumor cells.[11] The molecular and cellular cascades that allow for the emission of immunogenic signals by dying cancer cells—on one hand—and for their perception by the immune system—on the other hand—have been intensively investigated. A detailed discussion of these mechanistic aspects however exceeds the scope of this Trial Watch and can be found elsewhere.[3,16]

To date, a rather restricted number of stimuli has been shown to induce bona fide ICD, hence converting dying cancer cells into a vaccine that elicits protective tumor-specific immune responses.[3] These include some types of radiotherapy and photodynamic therapy,[12,17–19] cardiac glycosides[20,21] and a few chemotherapeutics: the DNA alkylating agent cyclophosphamide,[22] the anthracyclines doxorubicin and mitoxantrone,[5,23,24] the platinum derivative oxaliplatin,[25] the microtubular poison patupilone (also known as epothilone B or EPO906)[26] and the epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.[27] The histone deacetylase inhibitor vorinostat as well as the n3-polyunsaturated fatty acid docosahexaenoic acid have been shown to promote the exposure of CRT on brain, pancreatic and bladder cancer cell lines.[28,29] Along similar lines, bortezomib, an inhibitor of the proteasome nowadays approved by FDA for cancer therapy, reportedly stimulates the exposure of HSP90 on the surface of dying multiple myeloma cells,[30] and the oncolytic coxsackievirus B3 has been suggested to kill non-small cell lung carcinoma (NSCLC) cells while allowing for the exposure of CRT on the cell surface, the secretion of ATP and the release of HMGB1.[31] Finally, it has recently been demonstrated that neoplastic cells succumbing to shikonin, a phytochemical endowed with anti-inflammatory and antitumor properties, not only emits the three main signals associated with ICD abut also increase the immunogenic potential of DC-based vaccines.[32,33] However, none of these observations has been confirmed with appropriate vaccination assays in vivo (see below),[16] casting doubts on the hypothesis that vorinostat, docosahexaenoic acid, bortezomib, coxsackievirus B3 and shikonin would constitute bona fide ICD inducers. In 2012, we have demonstrated that hyperploidy, be it spontaneous or triggered by cytoskeletal poisons such as cytochalasin D or dihydrocytochalasin B, is associated with increased baseline levels of CRT exposure on the cell surface.[26] Although this underpins an immunosurveillance mechanism that controls the ploidy of cancer cells in vivo, neither of these chemicals were shown to induce bona fide ICD, at least at the hyperploidizing concentrations employed in our study.[26]

Importantly, there appears to be no simple structure-function relationship between chemotherapeutics and their capacity to induce ICD, as chemically similar agents such as cisplatin and oxaliplatin do not behave similarly in this sense.[25,34] This implies that the capacity of a given chemical to promote ICD cannot be predicted based on its structural analogy with a known ICD inducer, but rather must be assessed experimentally. We have recently developed fluorescent biosensors to monitor CRT exposure, ATP secretion and HMGB1 release in a high-throughput setting, allowing for the relatively straightforward identification of chemicals that induce the major hallmarks of ICD.[20] Nevertheless, the true potential of a given stimulus to promote ICD must be confirmed in vivo, in assays that specifically evaluate the capacity of dying cancer cells to induce a protective immune response against live cells of the same type.[3]

Along the lines of our monthly Trial Watch series,[35–45] here we will summarize the latest advances in the use of bona fide ICD inducers as anticancer agents, focusing on high-impact studies that have been published and clinical trials that have been launched during the last 13 months. Of note, the approval status of these agents has not varied since the publication of the latest Trial Watch dealing with this topic.[44] Thus, (1) cyclophosphamide, doxorubicin and oxaliplatin are nowadays approved by FDA and other regulatory agencies for the treatment of distinct hematologic and solid malignancies, (2) mitoxantrone is predominantly employed for a cancer-unrelated indication, multiple sclerosis, even though mitoxantrone-containing chemotherapeutic regimens have also been endorsed by FDA for use in patients affected by acute myeloid leukemia, non-Hodgkin's lymphoma, breast and prostate carcinoma and (3) patupilone remains an investigational agent (**Table 1**).

Of note, 7A7 is a murine IgG_1 originally raised against murine EGFR, though it displays a consistent degree of cross-reactivity against human EGFR.[46] Since multiple agents that specifically target human EGFR are currently available, including FDA-approved molecules (e.g., the tyrosine kinase inhibitor erlotinib,

the monoclonal antibody cetuximab)[47–49] as well as a wealth of investigational compounds (e.g., the monoclonal antibodies necitumumab and nimotuzumab),[38,41] it seems very unlikely that the development of 7A7 will ever be pursued until a clinical stage. Unfortunately, for the reasons discussed above, no predictions can be put forward on the ability of other EGFR-targeting agents to stimulate bona fide ICD.[50]

Literature Update

Since the submission of our previous Trial Watch on this topic (December 2011),[44] high-impact journals (latest impact factor > 15, according to Thomson Reuters–ISI Web of Knowledge) dealing with clinical cancer research (i.e., *Science*, *Nature*, *Nature Medicine*, *The New England Journal of Medicine*, *Lancet*, *Lancet Oncology* and *The Journal of Clinical Oncology*) have published the results of no less than 100 clinical trials involving the use of ICD inducers in cancer patients. In the vast majority of cases, however, cyclophosphamide, doxorubicin, oxaliplatin and mitoxantrone were not evaluated for novel therapeutic indications but administered to the control arm of patient cohorts as part of gold standard therapeutic regimens. Thus, during the last 13 months, a relatively limited number of high-impact publications reported the results of clinical studies aimed at investigating the use of FDA-approved ICD inducers (mitoxantrone excluded) as off-label anticancer medications. Alongside, one single article dealt with the potential antineoplastic activity of patupilone (source www.ncbi.nlm.nih.gov/sites/entrez/).

Combinatorial chemotherapeutic regimens involving metronomic cyclophosphamide have been tested in two Phase I clinical trials enrolling cancer patients of different ages.[51,52] Thus, the combination of metronomic cyclophosphamide with the poly(ADP-ribose) polymerase (PARP) inhibitor velaparib turned out to be well tolerated by adults affected by refractory solid tumors and to exert promising antineoplastic effects in a subset of patients bearing *BRCA1/2* mutations.[51] Along similar lines, low-dose cyclophosphamide combined with the vascular endothelial growth factor (VEGF)-specific monoclonal antibody bevacizumab[53] and the FDA-approved multi-kinase inhibitor sorafenib[54] induced no significant toxicity (and exerted antineoplastic activity, at least to some extent) in children and young adults affected by refractory/recurrent solid tumors.[52] In addition, it has recently been shown that metronomic cyclophosphamide efficiently reduce the number of immunosuppressive $FOXP3^+$ regulatory T cells in the circulation of advanced renal cell carcinoma patients, allowing for the development of antitumor responses upon the administration of a multipeptide vaccine formulation (IMA901).[55]

As an off-label anticancer agent, doxorubicin has been recently tested (1) in combination with the cyclin-dependent kinase (CDK) inhibitor flavopiridol in patients affected by advanced sarcomas,[56] (2) as a non-targeted liposomal formulation and combined with bevacizumab plus temsirolimus (an inhibitor of the mammalian target of rapamycin, mTOR, approved by FDA for use in renal cell carcinoma patients)[57] in individuals bearing advanced malignancies[58] and (3) as the chemotherapeutic cargo of EGFR-targeted immunoliposomes in patients affected by advanced solid tumors.[59] All these doxorubicin-based chemotherapeutic regimens were well tolerated and exerted (at least partial) antineoplastic effects, supporting the initiation of Phase II studies. Furthermore, the combination of doxorubicin, cisplatin and etoposide (a topoisomerase II inhibitor) has been shown to ameliorate the response rate and progression-free survival (but not the overall survival) of advanced adrenocortical carcinoma patients receiving mitotane (a derivative of the organochloride insecticide DDT currently approved by FDA for use in these patients) more consistently than streptozocin (a natural alkylating agent nowadays employed against pancreatic cancer).[60]

The most remarkable progress in the clinical development of oxaliplatin achieved since the submission of our latest Trial Watch (December 2011)[44] surely involve pancreatic cancer.[61–63] The FOLFIRINOX regimen (folinic acid + 5-fluorouracil + irinotecan + oxaliplatin) has indeed been shown not only to increase the response rate but also to extend the overall survival (by more than 4 months) of individuals affected by metastatic pancreatic cancer, as compared with the nucleoside analog gemcitabine.[61–64] Although this chemotherapeutic regimen was also associated with an increased rate of adverse events,[62] it has generated a considerable clinical interest, as demonstrated by the consistent number (namely, 21) of clinical trials initiated during the last 13 months to test the antineoplastic profile of oxaliplatin-based chemotherapy in cohorts of pancreatic cancer patients (see below). Very encouraging results have been obtained in two Phase III clinical trials testing (1) the XELOX (capecitabine + oxaliplatin) regimen as an adjuvant intervention for patients subjected to curative D2 gastroctemy[65] and (2) the GEMOX (gemcitabine + oxaliplatin) regimen, as a standalone intervention or combined with erlotinib, in patients affected by biliary-tract cancer.[66] In line with this notion, no less than 21 clinical trials have recently been initiated to test the antineoplastic potential of oxaliplatin-based chemotherapeutic regimens in cohorts of patients affected by gastric, gastresophageal, gastrointestinal and biliary duct neoplasms.

During the last 13 months, high-impact journals have published one single article dealing with the clinical anticancer potential of patupilone. In this work, Colombo and colleagues report the results of a randomized, open-label, Phase III study comparing patupilone with pegylated liposomal doxorubicin (PLD) in platinum-refractory patients affected by recurrent ovarian carcinoma, primary fallopian tube cancer or primary peritoneal cancer.[67] A total of 829 individuals were randomly assigned to receive either 10 mg/m^2 patupilone (i.v., every 3 weeks) or 50 mg/m^2 PLD (i.v., every 4 weeks) and monitored for clinical progression and the emergence of adverse effects. Of note, although a higher rate of partial responses was recorded in the experimental arm, patupilone did not significantly ameliorate overall survival (the primary end-point of the study) as compared with PLD.[67]

Focusing on recent, high-impact translational research focusing on ICD-inducing chemotherapeutics, we have found of particular interest the works by Senovilla et al., Dewan et al., Qayum et al. and Zhang et al., demonstrating (1) that patupilone is capable of triggering ICD;[26] (2) that low-dose cyclophosphamide and irradiation (both constituting bona fide ICD inducers) synergize with the FDA-approved TLR7 agonist imiquimod in inhibiting

Table 2. Clinical trials recently launched to evaluate the antineoplastic profile of cyclophosphamide as an off-label medication*

Indications	Status	Phase	Notes	Ref.
CML MDS Solid tumors	Not yet recruiting	n.a.	Combined with busulfan and followed by allogeneic HSCT	NCT01685411
		II	Combined with fludarabine, TBI and followed by UCBT	NCT01690520
	Recruiting	I/II	Combined with fludarabine, TBI and followed by HSCT	NCT01509300
Ewing's sarcoma Neuroblastoma	Recruiting	II	Combined with topotecan and bevacizumab	NCT01492673
HNC	Recruiting	II	Combined with cetuximab	NCT01581970
Melanoma	Not yet recruiting	I/II	Combined with fludarabine, ACT and rIL-2	NCT01740557
	Recruiting	n.a.	Combined with fludarabine, ACT, rIL-2 and ipilimumab	NCT01701674
		I	Combined with fludarabine, ACT, rIL-2 and vemurafenib	NCT01585415
			Combined with ACT and vemurafenib	NCT01659151
		II	Combined with fludarabine, ACT and rIL-2	NCT01495572
			Combined with ipilimumab	NCT01740401
Mesothelioma Pancreatic cancer	Recruiting	II	Combined with fludarabine and ACT with anti-mesothelin PBLs	NCT01583686
Pancreatic cancer	Recruiting	n.a.	FOLFIRINOX regimen combined with SBRT and tumor-cell vaccine	NCT01595321
Pediatric solid tumors	Not yet recruiting	n.a.	As single agent	NCT01661400
		II	Combined with carboplatin, etoposide, irinotecan and vincristine	NCT01535183
	Recruiting	I	Combined with rapamycin and topotecan	NCT01670175
Prostate cancer	Recruiting	I/II	Combined with androgen ablation and a GM-CSF-expressing tumor-cell vaccine	NCT01696877
Rectal carcinoma	Recruiting	II	Combined with chemoradiotherapy and a MUC1-targeting vaccine	NCT01507103
Reproductive tract cancers	Recruiting	I	Combined with a FRα-targeting vaccine	NCT01606241
Sarcoma	Recruiting	II	As single agent	NCT01716689
Solid tumors	Recruiting	I	Combined with an oncolytic virus	NCT01598129
		II	Combined with fludarabine, irradiation, ACT and rIL-2	NCT01697527

Abbreviations: ACT, adoptive cell transfer; CML, chronic myelogenous leukemia; FOLFIRINOX, folinic acid, 5-fluorouracil, irinotecan, oxaliplatin; FRα, folate receptor α; GM-CSF, granulocyte-macrophage colony-stimulating factor; HNC, head and neck cancer; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; MUC1, mucin 1; n.a., not available, rIL-2, recombinant interleukin-2; PBL, peripheral blood lymphocytes; SBRT, stereotactic body radiation; TBI, total body irradiation; UCBT, umbilical cord blood transplantation. *between December 1, 2011, and the day of submission.

tumor growth in an ectopic murine model of breast carcinoma, an effect that is associated with abundant tumor infiltration by $CD11c^+$, $CD4^+$ and $CD8^+$ cells; (3) that a chemical inhibitor of Class I phosphoinositide-3-kinases (i.e., GDC-0941) normalizes the tumor vasculature in vivo, hence increasing perfusion, restoring normoxia and allowing for the delivery of increased amounts of doxorubicin;[68] and (4) that doxorubicin—contrarily to long-established convictions—does not exert cardiotoxic effects as it directly stimulates the mitochondrial generation of reactive oxygen species (ROS), but rather by specifically targeting topoisomerase IIβ (at least in mice).[69]

Update on Clinical Trials

When this Trial Watch was being redacted (December 2012), official sources listed no less than 305 clinical trials launched after December 1, 2011, that would investigate the safety and therapeutic profile of bona fide ICD inducers in cancer patients (source www.clinicaltrials.gov). Of these, 83 studies are based on (or at least include among other therapeutic interventions) cyclophosphamide, 63 doxorubicin, 80 oxaliplatin and 9 mitoxantrone. Of note, no clinical trials involving patupilone have been registered at www.clinicaltrials.gov during the last 13 months. An unrestricted search for the term "patupilone" returns 59 entries; yet only 29 of these actually involved patupilone. Virtually all clinical studies testing patupilone in cancer patients have nowadays been completed, suspended, withdrawn or terminated, as they were near-to-invariably initiated before 2008.

Among 83 recently initiated clinical trials investigating the safety and therapeutic profile of cyclophosphamide in cancer patients, 61 involve only indications for which this drug has already been approved by FDA (**Table 1**), including breast cancer

Table 3. Clinical trials recently launched to evaluate the antineoplastic profile of doxorubicin as an off-label medication*

Indications	Status	Phase	Notes	Ref.
Advanced or metastatic solid tumors	Not yet recruiting	II	Combined with HIFU	NCT01640847
	Recruiting	I/II	Combined with aldoxorubicin	NCT01673438
		III	As liposomal injection combined with carboplatin and iniparib	NCT01593228
CML	Not yet recruiting	II	Combined with various drugs including araC, folonic acid and metotrexate	NCT01670084
Bladder cancer Urothelial cancer	Not yet recruiting	II	MVAC regimen combined with G-CSF	NCT01639521
Colorectal carcinoma	Not yet recruiting	II	As single agent	NCT01703910
Hepatic metastases of neuroendocrine tumors	Not yet recruiting	II	Combined with chemoembolization and everolimus	NCT01678664
Leiomyosarcoma	Recruiting	III	TAC regimen combined with gemcitabine and G-CSF	NCT01533207
Sarcoma	Not yet recruiting	n.a.	Combined with various drugs including dexamethasone and vincristine	NCT01490060
	Recruiting	I	Combined with bevacizumab and radiotherapy	NCT01746238
Reproductive tract cancers	Not yet recruiting	I	As PLD and combined with EGEN-001	NCT01673477
	Recruiting	I	As PLD and combined with EGEN-001	NCT01489371
		II	Combined with cisplatin	NCT01659554
			As PLD and combined with TLR8 agonist	NCT01666444

Abbreviations: araC, cytarabine; CML, chronic myelogenous leukemia; G-CSF, granulocyte colony-stimulating factor; HIFU, high intensity focused ultrasound; MVAC, cisplatin, vinblastine, doxorubicin, methotrexate; n.a., not available; PLD, pegylated liposomal doxorubicin; TAC, docetaxel, doxorubicin, cyclophosphamide; TLR8, Toll-like receptor 8. *between December 1, 2011, and the day of submission.

(23 trials) and a wide panel of hematological malignancies (38 trials) (source www.clinicaltrials.gov). In addition, cyclophosphamide is currently being tested in patients affected by: (1) melanoma (NCT01495572; NCT01585415; NCT01659151; NCT01701674; NCT01740401; NCT01740557), in combination with adoptive cell transfer, or the FDA-approved anti-cytotoxic T-lymphocyte antigen 4 (CTLA4) monoclonal antibody ipilimumab;[70,71] (2) pediatric solid (including brain) tumors (NCT01535183; NCT01661400; NCT01670175), as a standalone intervention or combined with either conventional chemotherapeutic regimens or rapamycin (another immunosuppressive inhibitor of mTOR)[72] plus topotecan (a topoisomerase inhibitor); (3) hematological malignancies (NCT01509300; NCT01685411; NCT01690520), invariably as part of a lymphodepleting/lymphoablative conditioning regimen that precedes hematopoietic stem cell transplantation; (4) sarcoma (NCT01492673; NCT01716689), as a single agent or in association with topotecan and bevacizumab; (5) head and neck cancer (NCT01581970), in combination with cetuximab;[49] (6) mesothelioma and pancreatic cancer (NCT01583686), as a part of a conditioning regimen followed by the administration of peripheral blood lymphocytes engineered to express an anti-mesothelin chimeric antigen receptor (CAR);[73–75] (7) pancreatic cancer (NCT01595321), as an immunostimulatory intervention to maximize the efficacy of a tumor-cell vaccine; (8) prostate cancer (NCT01696877), combined with androgen ablation and a granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transfected tumor-cell vaccine; (9) rectal carcinoma (NCT01507103), in association with chemotherapy and a liposomal vaccine that targets the exposed core domain of mucin 1 (L-BLP25); (10) reproductive tract neoplasms (NCT01606241), as an immunostimulatory intervention in support of a folate receptor α (FRα)-targeting vaccine; and (11) not better defined advanced solid tumors (NCT01598129; NCT01697527), either as part of a lymphodepleting regimen preceding the infusion of genetically engineered lymphocytes or combined with an oncolytic adenovirus (CGTG-02) (**Table 2**).

Forty-nine out of 63 clinical studies recently launched to evaluate the safety profile and antineoplastic activity of doxorubicin in cancer patients involve only indications for which this compound is nowadays approved by FDA (**Table 1**), including breast carcinoma (15 trials), hepatocellular carcinoma (6 trials), ovarian carcinoma (3 trials), soft tissue sarcoma (2 trials), pancreatic carcinoma (1 trial) and a wide array of hematological tumors (22 trials) (source www.clinicaltrials.gov). Furthermore, doxorubicin is being assessed as an antineoplastic agent in cohorts of patients bearing: (1) neoplasms of the reproductive tract (NCT01489371; NCT01659554; NCT01666444; NCT01673477), most often as PLD combined with cisplatin or with immunostimulatory interventions encompassing TLR8 agonists and an IL-12-encoding plasmid; (2) sarcomas or leiomyosarcomas (NCT01490060; NCT01533207; NCT01746238), in combination with conventional chemotherapeutic regimens, bevacizumab plus radiotherapy or gemcitabine plus granulocyte colony-stimulating factor (G-CSF); (3) chronic myelogenous leukemia (NCT01670084), as a part of a combinatorial therapy involving—among other drugs—cytarabine (a nucleoside analog), corticosteroids and methotrexate (an antifolate); (4) bladder and urothelial cancer (NCT01639521), combined with cisplatin, vinblastine (a microtubular poison) and methotrexate; (5) metastatic

Table 4. Clinical trials recently launched to evaluate the antineoplastic profile of oxaliplatin as an off-label medication*

Indications	Status	Phase	Notes	Ref.
BCL	Active, not recruiting	I/II	GEMOX regimen plus rituximab	NCT01562990
	Recruiting	III	GEMOX regimen	NCT01670370
Biliary tract cancer	Recruiting	II	FOLFIRINOX regimen	NCT01494363
Biliary tract cancer Gastrointestinal cancer Pancreatic cancer	Recruiting	I	FOLFIRINOX regimen	NCT01643499
Breast carcinoma	Recruiting	II	FOLFOX regimen plus bevacizumab	NCT01658033
			NVBOX regimen	NCT01528826
Cholangiocarcinoma	Recruiting	n.a.	GEMOX regimen plus dexamethasone plus floxuridine	NCT01525069
		II	FOLFOX regimen plus capecitabine	NCT01572324
Gastric cancer	Not yet recruiting	II	XELOX regimen	NCT01665274
			XELOX regimen followed by docetaxel	NCT01558011
		III	SOX or XELOX regimen	NCT01534546
			SOX regimen	NCT01671449
	Recruiting	II	Adjuvant XELOX regimen	NCT01618474
			EOX regimen plus immunotherapy	NCT01630083
			FOLFOX regimen plus onartuzumab	NCT01590719
			SOX regimen	NCT01552980
		II/III	Adjuvant or perioperative SOX or XELOX regimen	NCT01516944
		III	Adjuvant or neoadjuvant SOX regimen	NCT01583361
			DOS regimen	NCT01515748
			FOLFOX regimen plus onartuzumab	NCT01662869
			XELOX regimen plus radiotherapy	NCT01711242
Gastresophageal cancers	Not yet recruiting	II	FOLFOX regimen plus anti-VEGF therapy	NCT01747551
	Recruiting	I	XELOX regimen	NCT01719926
		II	Combined with radiotherapy and raltiterxed	NCT01732380
			FOLFOX regimen	NCT01498289
		II/III	Combined with docetaxel, 5-FU and radiotherapy	NCT01523015
Gastrointestinal tumors	Recruiting	I	OIS regimen	NCT01693445
		II	SOX regimen	NCT01608646

Abbreviations: 5-FU, 5-fluorouracil; BCL, B-cell lymphoma; DOS, docetaxel + oxaliplatin; EOX, epirubicin + oxaliplatin + capecitabine; FOLFIRINOX, folinic acid + 5-FU + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-FU + oxaliplatin; GEMOX, gemcitabine + oxaliplatin; n.a., not available; NVBOX, vinorelbine + oxaliplatin; OIS, oxaliplatin + irinotecan + S-1; SOX, S-1 + oxaliplatin; VEGF, vascular endothelial growth factor; XELOX, capecitabine + oxaliplatin. *between December 1, 2011, and the day of submission.

colorectal carcinoma (NCT01703910), as a standalone intervention; (6) hepatic metastases from digestive endocrine neoplasms (NCT01678664), in the context of chemoembolization and followed by the administration of everolimus (an immunosuppressive derivative of rapamycin); and (7) not better defined advanced or metastatic solid tumors (NCT01593228; NCT01640847; NCT01673438), in the form of both free and tumor-targeted drug, combined with carboplatin (a platinum compound related, but not equivalent to, cisplatin and oxaliplatin)[25,76,77] plus iniparib (a compound originally developed as—but later disconfirm from being—an irreversible inhibitor of PARP1),[78,79] or coupled to high intensity focused ultrasound (HIFU) (**Table 3**).

Among 80 clinical trials recently launched to estimate the safety and efficacy of oxaliplatin in cancer patients, 30 are enrolling/will enroll only individuals affected by colorectal carcinoma, the sole indication for which this platinum-derivative is currently approved by FDA and other international regulatory agencies (**Table 1**) (source www.clinicaltrials.gov). In addition, oxaliplatin is currently being tested for its antineoplastic properties in cohorts of patients bearing: (1) pancreatic cancer (NCT01485744; NCT01521702; NCT01524575; NCT01526135; NCT01558869; NCT01560949; NCT01581307; NCT01586611; NCT01591733; NCT01595321; NCT01632306; NCT01643499; NCT01652976; NCT01658943; NCT01660711; NCT01666730; NCT01671202; NCT01683422; NCT01688336; NCT01744353; NCT01571024), near-to-invariably as part of conventional GEMOX, FOLFOX (folinic acid + 5-fluorouracil + oxaliplatin) or FOLFIRINOX regimens; (2) gastric, gastresophageal or gastrointestinal tumors (NCT01515748; NCT01516944; NCT01534546; NCT01552980; NCT01558011;

Table 4 (Continued). Clinical trials recently launched to evaluate the antineoplastic profile of oxaliplatin as an off-label medication*

Indications	Status	Phase	Notes	Ref.
Pancreatic cancer	Not yet recruiting	I	FOLFOX regimen plus PI3K inhibitor	NCT01571024
		I/II	FOLFOX regimen plus LY2090314	NCT01632306
				NCT01671202
		II	FOLFOX regimen plus metformin	NCT01666730
		III	FOLFOX regimen	NCT01586611
		n.a.	FOLFIRINOX regimen combined with SBRT and tumor-cell vaccine	NCT01595321
	Recruiting	I	FOLFIRINOX plus LDE225	NCT01485744
			FOLFOX-A regimen	NCT01744353
			GEMOX regimen	NCT01524575
			XELOX regimen plus irinotecan	NCT01558869
			Neoadjuvant FOLFIRINOX regimen followed by gemcitabine-based therapy	NCT01560949
		II	FOLFOX regimen plus theraspheres	NCT01581307
			FOLFIRINOX regimen followed by capecitabine and radiotherapy	NCT01591733
			FOLFOX regimen + dasatinib	NCT01652976
			FOLFOX regimen	NCT01658943
			FOLFIRINOX regimen	NCT01660711
			XELOX regimen plus gemcitabine, erlotinib and proton radiation	NCT01683422
			Neoadjuvant GEMOX regimen	NCT01521702
		III	FOLFIRINOX regimen	NCT01526135
				NCT01688336
Peritoneal carcinomatosis	Recruiting	II	As normothermic or hypothermic intraperitoneal chemotherapy	NCT01575730
Solid tumors	Recruiting	I/II	FOLFOX regimen plus tivantinib	NCT01611857

Abbreviations: FOLFIRINOX, folinic acid + 5-FU + irinotecan + oxaliplatin; FOLFOX, folinic acid + 5-FU + oxaliplatin; FOLFOX, folinic acid + 5-FU + oxaliplatin + abraxane; GEMOX, gemcitabine + oxaliplatin; n.a., not available; PI3K, phosphoinositide-3-kinase; SBRT, stereotactic body radiation therapy; XELOX, capecitabine + oxaliplatin. *between December 1, 2011, and the day of submission.

NCT01583361; NCT01590719; NCT01618474; NCT01630083; NCT01662869; NCT01665274; NCT01671449; NCT01711242; NCT01643499; NCT01608646; NCT01693445; NCT01498289; NCT01523015; NCT01732380; NCT01747551; NCT01719926), most often as part of established FOLFOX, DOS (docetaxel + oxaliplatin), SOX (S-1 + oxaliplatin) or XELOX regimens; (3) B-cell lymphoma (NCT01562990, NCT01670370), in the context of the GEMOX treatment alone or further combined with the FDA-approved anti-CD20 monoclonal antibody rituximab;[80,81] (4) breast carcinoma (NCT01528826; NCT01658033), combined with vinorelbine (a vinca alkaloid) or in the context of the FOLFOX regimen plus bevacizumab; (5) cholangiocarcinoma (NCT01525069; NCT01572324), combined with gemcitabine, dexamethasone and floxuridine (a nucleoside analog) or as part of the FOLFOX treatment plus capecitabine; (6) biliary tract neoplasms (NCT01494363; NCT01643499), as part of the FOLFIRINOX regimen; (7) peritoneal carcinomatosis (NCT01575730), as a standalone chemotherapeutic intervention, given as normothermic vs. hyperthermic intraoperative chemoperfusion; and (8) not better specified solid tumors (NCT01611857), in the context of the FOLFOX treatment further combined with tivantinib (a hitherto experimental inhibitor of the oncogenic kinase MET)[82] (**Table 4**).

Of 9 clinical studies initiated after 2011, December 1 to assess the safety and efficacy of mitoxantrone in cancer patients, 6 involve only indications for which this anthracycline is currently approved by FDA (**Table 1**), including various types of leukemia (4 trials) and prostate cancer (2 trials) (source www.clinicaltrials.gov). Moreover, mitoxantrone is nowadays being investigated in patients affected by (1) myelodysplastic syndromes (NCT01701375; NCT01729845), combined with cytarabine and either decitabine (a hypomethylating agent) plus etoposide or an experimental inhibitor of CDK4 and CDK6 (PD 0332991);[83] and (2) relapsed or refractory mantle cell lymphoma (NCT01578343), as a part of a combinatorial regimen including fludarabine (a nucleoside analog), vorinostat (an inhibitor of histone deacetylases) and corticosteroids (dexamethasone) (**Table 5**).

Concluding Remarks

The concept of immunogenic cell death has originally been proposed in 2005 by Casares et al., who were the first (1) to demonstrate that malignant cells exposed to anthracyclines are capable of vaccinating mice against a subsequent challenge with tumor cells of the same type, and (2) to provide mechanistic insights into this phenomenon, i.e., to show that it depends on the activity

Table 5. Clinical trials recently launched to evaluate the antineoplastic profile of mitoxantrone as an off-label medication*

Indications	Status	Phase	Notes	Ref.
MDS	Not yet recruiting	I/II	Combined with araC, decitabine and etoposide	NCT01729845
	Recruiting	I	Combined with araC and PD 0332991	NCT01701375
MCL	Not yet recruiting	II	Combined with dexamethasone, fludarabine and vorinostat	NCT01578343

Abbreviations: araC, cytarabine; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome. *between December 1, 2011, and the day of submission.

of caspases.[23] Since then, the molecular machineries whereby dying cancer cells emit a spatiotemporally defined combination of signals that—at least under selected circumstances—can be translated by the immune system into an antitumor response have been intensively investigated.[5,6,10,12,20] Alongside, the list of bona fide ICD inducers has been significantly expanded, though at a relatively slow pace. One of the major obstacles to the identification of novel chemicals that trigger bona fide ICD is that this property cannot be easily predicted starting from structural features, but rather must be tested experimentally. As a standalone example, cisplatin and oxaliplatin, two structurally related platinum derivatives that ignite largely redundant (though not precisely overlapping) signaling pathways and activate highly similar (though not identical) resistance mechanisms,[76] differ in their capacity to induce ICD.[25,34] In addition, the immunogenic potential of some chemotherapeutics is largely influenced by dose and administration schedule. Thus, while high-dose cyclophosphamide is well known for its potent immunosuppressive activity, and has been used for years in this sense (for instance as part of lymphodepleting/lymphoablative regimens to condition transplantation recipients), the same drug administered metronomically exerts a wide array of immunostimulatory functions.[22,44,84,85] Thus, even though high-throughput screening-compatible systems allowing for the evaluation of the major hallmarks of ICD (i.e., CRT exposure, ATP secretion, HMGB1 release) are nowadays available,[20] the gold standard method to assess the ability of a given stimulus to trigger ICD is constituted by vaccination assays in immunocompetent, syngenic animals.[3,16] Besides being cost-ineffective and relatively time-consuming, this approach turns out to be particularly disadvantageous when murine cells are resistant to experimental manipulations that "normally" affect their human counterparts, e.g., cardiac glycosides.[20,21,39] It is tempting to speculate, yet remains to be formally demonstrated, that the use of mice genetically engineered to bear a virtually complete human immune system (so-called humanized mice)[86] may circumvent—at least in part—this issue.

One of the most recently identified ICD inducers is patupilone,[26] a macrolide of the epothilone family originally described in 1995 for its ability to stabilize microtubules similar to taxol.[87–89] The synthesis and preclinical characterization of epothilones generated an intense wave of enthusiasm,[90,91] rapidly translating into dozens of clinical studies.[92–95] In 2007, the U.S. FDA approved ixabepilone (a derivative of patupilone) for use as a standalone intervention in anthracycline-, taxane- and capecitabine-resistant breast carcinoma patients, or combined with capecitabine in patients bearing anthracycline- and taxane-resistant locally advanced or metastatic breast carcinoma.[96,97] This perhaps explains why the clinical interest in patupilone appears to have nowadays completely declined (see above). Unfortunately, to the best of our knowledge, whether ixabepilone also constitutes a bona fide ICD inducer has not yet been determined, nor it has been investigated to which extent the immune system contributes to the clinical efficacy of this epothilone. Future studies will have to address these two therapeutically relevant questions.

In addition, it will be crucial to identify (1) therapeutic interventions that convert non-immunogenic apoptosis into ICD, as well as (2) biomarkers that can be used to predict the propensity of the patient's immune system to productively detect ICD and translate it into an anticancer immune response. Some progress has already been made in this sense. On one hand, it has been demonstrated that cisplatin-induced cell death, which per se is associated with the secretion of ATP and the release of HGMB1 but not with the exposure of CRT on the cell surface, can be rendered immunogenic by the adsorption of recombinant CRT on dying cells as well as by the co-administration of the ER stressors such as thapsigargin and tunicamycin.[5,34] Along similar lines, autophagy-deficient cancer cells succumbing to anthracyclines (i.e., bona fide ICD inducers) are intrinsically unable to promote ICD as they do not release ATP.[9,10] At least speculatively, this represents a clinically unfavorable situation, which nevertheless can be reverted by the local administration of extracellular ATPase inhibitors such as suramine.[10] On the other hand, breast carcinoma patients bearing loss-of-function polymorphisms in *TLR4* and *P2RX7* have been shown to relapse more quickly upon anthracycline-based chemotherapy and radiotherapy than control patients bearing wild-type polymorphisms.[11,15]

As it stands, the capacity of a given chemotherapeutic agent to promote ICD is only the first conditio sine qua non for the elicitation of therapeutic anticancer immune response. In addition, (1) the molecular machinery underpinning the emission of immunogenic signals by dying cancer cells must fully functional and (2) the patient's immune system must be able to detect these signals, decode them and eventually orchestrate a cytotoxic T-cell response. Investigating in detail these three "modules" (i.e., the ICD inducer, the cancer cell and the patient's immune system) and how they mutually interact will undoubtedly drive the discovery of novel ICD inducers as well as of clinically meaningful strategies to endow originally non-immunogenic instances of cell death with potent immunogenic properties.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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