



HAL
open science

Preconditioning with immunogenic cell death-inducing treatments for subsequent immunotherapy

Hui Pan, Peng Liu, Guido Kroemer, Oliver Kepp

► **To cite this version:**

Hui Pan, Peng Liu, Guido Kroemer, Oliver Kepp. Preconditioning with immunogenic cell death-inducing treatments for subsequent immunotherapy. International review of cell and molecular biology / Int Rev Cell Mol Biol, 2024, International Review of Cell and Molecular Biology, 382, pp.279-294. 10.1016/bs.ircmb.2023.06.001 . hal-04601417

HAL Id: hal-04601417

<https://hal.science/hal-04601417>

Submitted on 4 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Preconditioning with immunogenic cell death-inducing treatments for subsequent immunotherapy

Hui Pan^{1,2,3}, Peng Liu^{1,2}, Guido Kroemer^{1,2,4}, Oliver Kepp^{1,2}

¹Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, Inserm U1138, Institut Universitaire de France, Paris, France.

²Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France.

³Faculté de Médecine, Université de Paris Saclay, Kremlin Bicêtre, France.

⁴Institut du Cancer Paris CARPEM, Department of Biology, Hôpital Européen Georges Pompidou, AP-HP, Paris, France.

Correspondence to: Guido Kroemer (kroemer@orange.fr); Oliver Kepp (captain.olsen@gmail.com); Peng Liu (peng.liu@inserm.fr)

Keywords: Immunotherapy, cancer, adjuvanticity, PD-1; PD-L1; CTLA-4

Wordcount: 5827

Conflict of interest statement: The authors declare no potential conflicts of interest

Abstract

Since the dawn of anticancer immunotherapy, the clinical use of immune checkpoint inhibitors (ICI) has increased exponentially. Monoclonal antibodies targeting CTLA-4 and the PD-1/PD-L1 interaction were first introduced for the treatment of patients with unresectable melanoma. In melanoma, ICI lead to durable regression in a significant number of patients and have thus been clinically approved as a first-line treatment of advanced disease. Over the past years an increasing number of regulatory approvals have been granted for the use of ICI in patients affected by a large range of distinct carcinomas. In retrospect surprisingly, it has been discovered that particularly successful chemotherapeutic treatments are able to trigger anticancer immune responses because they induce immunogenic cell death (ICD), hence killing cancer cells in a way that they elicit an immune response against tumor-associated antigens. Logically, preclinical studies as well as clinical trials are currently exploring the possibility to combine ICD inducers with ICI to obtain optimal therapeutic effects. Here, we provide a broad overview of current strategies for the implementation of combinatorial approaches involving ICD induction followed by ICI in anticancer therapy.

Immunotherapy

Numerous strategies for the therapeutic (re)establishment of cancer immunosurveillance have been explored in the past, and an ever-increasing number of immunotherapies against cancer are being introduced into clinical routine at this time. Early immunotherapeutic treatments such as the non-specific immune stimulation by bacterial cell infiltrates employed by Coley in the late 19th century preceded the advent of chemo- and radiotherapy in the 20th century. Only in the past few decades immunotherapeutic approaches have been further explored and clinically employed, starting with the use of high-dose interferons or IL2 in patients with metastatic melanoma or renal cancer as well as the intravesical instillation of bacillus Calmette–Guérin in patients with superficial bladder cancer (1-3). Moreover, some conventional anticancer chemotherapeutics have been shown to induce immunogenic cell stress and death (ICD), thus stimulating adaptive immune responses against residual malignant cells (4).

Immune checkpoint inhibitors

The identification of immune checkpoints in the T cell compartment and the development of specific immune-checkpoint inhibitors (ICI) constituted a pivotal breakthrough for the immuno-oncology field, leading to their FDA approval for use in patients with metastatic melanoma. The first ICI to be FDA approved was ipilimumab, a human monoclonal antibody that inhibits the binding of cytotoxic T lymphocyte associated protein 4 (CTLA-4; CD152) to CD80 and CD86, followed by the later approval of the anti-programmed cell death protein 1 (PD-1; CD279) antibodies nivolumab and pembrolizumab (5, 6). Immune checkpoints enable the dynamic control of immune homeostasis and are particularly important for regulating T cell functionality. Thus, PD-1 and CTLA-4 are expressed on T cells, regulating T cell exhaustion and limiting the extent of T cell activation at the priming stage, respectively (7-10). The interaction of PD-1 with its ligand programmed death-ligand 1 (PD-L1; CD274), which is expressed on tumor and regulatory myeloid cells, induces a close-to-permanent dysfunctional state of T cell exhaustion characterized by limited cytotoxic effector function and a lack of response to stimuli, altogether maintaining immune tolerance (11). **(Fig. 1)** CTLA-4 mediates immunosuppression by interaction with the B7 proteins (CD80 and CD86) on the surface of antigen presenting cells, hence acting as a high affinity competitor of the costimulatory receptor CD28, thus restricting T cell activation at the priming stage (5, 10). These discoveries and their translational importance led to the award of the Nobel Prize in Physiology or Medicine in 2018 to James P. Allison and Tasuku Honjo for their pioneering research on CTLA-4 and PD-1, respectively (12). Meanwhile monoclonal antibodies targeting CTLA-4 and the PD-1/ PD-L1 axis have emerged as a core pillar of cancer therapy, which are approved for the treatment of a wide

range of cancers and have moved to frontline treatment in many advanced diseases such as melanoma, lung, kidney, liver, head and neck and triple-negative breast cancer (TNBC) (13). Currently there are eight FDA-approved ICI agents available for the treatment of 17 different malignancies, including the monoclonal antibodies pembrolizumab, nivolumab, cemiplimab and dostarlimab that target PD-1; atezolizumab, durvalumab and avelumab that target PD-L1; and ipilimumab which targets CTLA-4 (14, 15). Moreover, countless clinical trials are being managed or planned to expand the use of CTLA-4 and even more of PD-1/PD-L1 targeting antibodies for additional oncological indications.

Of note, acute clinical toxicities of common terminology criteria for adverse events grade ≥ 3 , as well as chronic effects that arise from long term exposure to ICI has limited their use in a large number of patients. In preclinical settings, the genetic ablation of CTLA-4 in mice induced more severe consequences early life, such as arthritis or cardiomyopathy, than the deletion of PD-1 or PD-L1, that caused less severe consequences at a much later stage (14, 16). Consistently, clinical experience shows an elevated incidence rate of high-grade toxicities in 38.6% - 57.9% of patients with advanced melanoma receiving anti-CTLA-4 and 10–15 % of patients treated with antibodies targeting PD-1 or PD-L1. Concurrent inhibition of CTLA-4 and PD-1/PD-L1 augments the risk of autoimmune toxicities, further increasing the percentage of high-grade immune-related adverse events (17).

Despite the successful implementation of ICI into clinical routine, with an estimated proportion of cancer patients eligible for ICI treatment of 36.1% in 2019, the response rate

estimated from a cross-sectional study of US patients is still limited to 12.5%. This is reflected by the highly variable magnitude of benefit for cancer patients with an odds ratio compared to conventional therapy ranging from 4.26 in metastatic melanoma down to 1 in colorectal cancer. This large degree of variation might be in part explained by differences in tumor mutational burden, mismatch repair deficiency, as well as by the immunological characteristics of the tumor microenvironment (TME), in particular the presence of tumor-infiltrating lymphocytes (TILs) before the initiation of the treatment (18).

Nonetheless, ICI targeting CTLA-4 and the PD-1/PD-L1 axis in cancer shows unprecedented efficacy in advanced-stage disease and has become a mainstay of modern medical oncology. Expanding immunotherapeutic approaches beyond ICI monotherapy is therefore a clear unmet clinical need. Here, we discuss strategies for the sequential combination of TME preconditioning plus ICI immunotherapy that may allow to limit the exposure to ICI while harnessing the synergistic therapeutic potential of such combination treatments.

Immunogenic cell death

A large number of oncological interventions have the ability to induce immunogenic cell death (ICD) of cancer cells, thus preconditioning the TME to subsequent immunotherapeutic treatments. Clinically actionable ICD inducers identified thus far comprise certain conventional chemotherapeutic agents including cyclophosphamide, anthracyclines, and platinum derivatives such as oxaliplatin and PT-112 (19-22). Moreover, other therapeutic approaches such as radiotherapy with ionizing irradiation, targeted anticancer agents, including bortezomib, crizotinib and ceritinib, as well as some variants of photodynamic therapy (PDT) and oncolytic virotherapy have been shown to induce ICD (23-26). In the course of ICD, malignant cells pass through a sequence of (terminal) cellular stress events, altogether increasing the antigenicity and adjuvanticity of the neoplastic lesion, eventually (re)instating cancer immunosurveillance (27-29). The onset of adaptive anticancer immunity is often associated with durable therapeutic success beyond treatment discontinuation.

Tumor antigens can be recognized by the host immune system and are continuously exploited by immunosurveillance mechanisms protecting the host from arising neoplasm. Thus, only failure of immunosurveillance leads to the establishment and later clinical manifestation of nascent tumors. In the course of ICD, malignant lesions re-establish antigenicity by stress-associated posttranslational changes in the immunopeptidome and the presentation of tumor associated antigens (TAA) as well as tumor neoantigens, oncofetal antigens or autoimmunity-prone lineage-specific antigens in autologous MHC class I complexes that can be recognized by the conventional $\alpha\beta$ T cell repertoire (30, 31). Moreover, ICD can concur with the expression of MHC molecules on tumor cells, thus

further enhancing their antigenic potential (32). Nevertheless, antigenicity itself is not sufficient to elicit adaptive immunity. In the absence of adjuvant and co-stimulatory signals, the presence of (auto)antigens usually results in T cell anergy and peripheral tolerance.

Cellular stress and death in the course of ICD trigger a series of endogenous adjuvant signals, cumulatively referred to as danger associated molecular patterns (DAMPs), expediting the attraction/activation and maturation of antigen presenting cells (APCs) (majorly dendritic cells, DCs), which facilitate tumor antigen presentation while delivering co-stimulatory signals both necessary for efficient T cell priming (33-38). In response to ICD induction, a set of spatiotemporally regulated DAMPs emitted by tumor cells are sensed by an evolutionarily conserved array of pattern recognition receptors (PRRs) expressed by myeloid cells (39).

The list of ICD-associated DAMPs includes ATP, which is secreted in a pannexin 1 (PANX1)- and lysosome-dependent way during the onset of autophagic stress or can be passively released when plasma membrane integrity is corrupted. Elevated levels of extracellular ATP bind to purinergic receptors such as P2YR2 expressed on immature APCs, resulting in their chemoattraction into the tumor bed (40). Final homing of APCs, in particular DCs, is guided by the liberation of the cytosolic protein annexin A1 (ANXA1) into the close vicinity of dying cancer cells. ANXA1 acts on formyl peptide receptor 1 (FPR1), which is expressed by myeloid cells including DCs (41). The relocation of endoplasmic reticulum (ER)-resident calreticulin (CALR) together with protein disulfide isomerase family A member 3 (PDIA3) to the surface plasma membrane of stressed and dying cancer cells occurs upon the onset of the unfolded protein response. CALR exposure

serves as an uptake signal that stimulates the phagocytosis of cancer cells by DCs, presumably through an interaction of CALR with LDL receptor-related protein 1 (LRP1) also known as apolipoprotein E receptor (APOER) or cluster of differentiation 91 (CD91) (42, 43). Moreover, type I interferon (IFN) responses (44) and the release of cancer cell-derived nucleic acids (45) triggered during ICD sustain adjuvant signaling and provide further APC stimuli. Altogether, ICD results in the onset of adaptive immune responses directing cytotoxic T lymphocytes against malignant cells in primary and metastatic lesions, accompanied by the education of memory T cells and the durable re-establishment of cancer immunosurveillance.

In sum, both adjuvanticity and antigenicity are indispensable features of ICD that together facilitate the onset of adaptive immunity and promote antitumor responses (33). Moreover, ICD causes the infiltration of tumors by immune cells including DCs and T lymphocytes and thus preconditions the TME for subsequent responses to ICI (**Fig. 2**).

Combinatorial approaches

In most patients, chemotherapy as well as ICI monotherapy fail to induce significant clinical responses. Primary resistance to ICI correlates with the absence of tumor-infiltrating lymphocytes (TILs) and is related to a general lack of pre-existing immune priming in the TME (46, 47). Consistently, scoring the density, type and localization of infiltrating immune cells, as well as measurements of the activation and exhaustion state of local T lymphocytes, are predictive of the response to ICIs (48, 49). Recently considerable efforts have been focused on approaches that turn “cold” tumors, which are scarcely infiltrated by leukocytes, into “hot” tumors, characterized by the local presence of immune effectors. This has been attempted by in situ vaccination with oncolytic viruses or physical treatments, such as fractionated radiotherapy, cryotherapy and targeted photodynamic therapy (50). Moreover, preclinical drug design now aims at targeting co-inhibitory receptors including lymphocyte activation gene 3 (LAG3), T cell immunoglobulin and mucin-domain containing 3 (TIM3), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain (TIGIT), V-domain immunoglobulin suppressor of T cell activation (VISTA), and B and T cell lymphocyte attenuator (BTLA), as well as modulating co-stimulatory signaling such as glucocorticoid-induced TNFR-related protein (GITR), tumor necrosis factor receptor superfamily, member 4 (TNFRSF4, better known as OX40 receptor), TNF receptor superfamily member 9 (4-1BB) and inducible T cell costimulator (ICOS) to reinvigorate T cell responses and further promote anticancer immunity.

Despite comparable clinical outcomes across many chemotherapy formulations the preconditioning of tumors via the stimulation of adaptive anticancer immunity through the

use of ICD-inducing regimens appears especially promising for the generation of synergistic therapeutic outcomes. Thus, combining ICD induction with additional immunotherapeutic interventions such as ICI targeting the PD-1/PD-L1 axis has already proven promising responses in preclinical models, as well as in clinical trials (51-54).

Preclinical ICD induction is generally performed several days prior to the administration of ICI in order to reshape the tumor microenvironment and to prime adaptive anticancer immunity. Thus, sequential combination of the ICD inducer oxaliplatin with PD-1/PD-L1 targeting ICI has shown synergistic interactions in several models of cancer (55-65). Genetic induction of Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation and tumor suppressor p53 deletion (KP) drives a model of non-small cell lung cancer (NSCLC) that exhibits a poor infiltration by CD3⁺ T cells at baseline and are notoriously resistant to treatment. However, KP NSCLC responded to a sequential combination of the ICD inducers oxaliplatin plus cyclophosphamide, followed by ICI targeting CTLA-4 and PD-1 (57). Consistently, the induction of ICD with targeted agents such as the tyrosine kinase inhibitor crizotinib can sensitize orthotopic models of NSCLC to subsequent immunotherapy with ICI in mice. Sequential combination of the ICD inducer crizotinib together with cisplatin, followed by PD-1 blockade cured 90% of the mice from established NSCLC (66, 67). In both studies, ICD induction yielded a significant infiltration of tumor nodules by CD3⁺ T cells as well as a rise in the ratio of tumor-infiltrating cytotoxic T lymphocytes (CTL) over immunosuppressive regulatory T cells (Tregs), indicative for the conversion of a 'cold' into a 'hot' TME susceptible to subsequent ICI. Synergistic effects of oxaliplatin-induced ICD exhibiting an increase in tumor infiltrating T cells prior to ICI-based immunotherapy have been confirmed in additional orthotopic models including

fibrosarcoma, colon, gastric and liver cancer (55-65). Oxaliplatin not only orchestrates the recruitment of CTLs to the site of the tumor but also leads to an enrichment of the TME with additional immune effectors and antigen presenting cells (APCs) such as natural killer (NK) cells and DC, respectively (56, 61, 68). Moreover, oxaliplatin triggers the depletion of immunosuppressive tumor-associated macrophages (TAM) and myeloid- derived suppressive cells (MDSC), thus provoking broad immunostimulatory effects while beneficially remodeling the TME (54, 61, 68). Other instances of ICD inducing regimens such as local oncolysis employing LTX- 401 or HER2-targeted antibody drug conjugates (ADC) with the anthracycline derivate T-PNU as payload showed synergistic effects with sequentially applied ICI and elicited long-lasting immune protection in murine models of orthotopic fibrosarcoma and breast cancer, respectively (69, 70). Altogether this indicates that preclinically ICD induction can be successfully employed for sensitizing tumors to subsequent ICI immunotherapy (**Fig. 3**).

Results from clinical studies investigating the sequential combination of ICD inducing regimens with subsequent ICI-based immunotherapy corroborate the hypothesis of beneficial ICD-mediated tumor preconditioning (52, 71, 72). Thus, ICD-inducing radiotherapy plus standard of care chemotherapy sensitized NSCLC patients to subsequent PD-L1 blockade, hence enhancing durable progression free survival (PFS) and overall survival (OS) compared to the placebo group that received chemoradiotherapy alone (73, 74). Similarly, immunogenic doxorubicin-based induction therapy in metastatic triple-negative breast cancer patients, followed by PD-1 blockade, increased the objective response rate from 20% (without induction therapy) to 35% (75). Moreover, the

anthracycline amrubicin was shown to sensitize small cell lung cancer (SCLC) to PD-1 targeting ICI, leading to an overall response rate (ORR) of 52% and a PFS at 1 year of 14.4% in patients with relapsed small cell lung cancer (SCLC) (76). In accord with preclinical data, the combination of oxaliplatin (which is an efficient ICD inducer) with ICI showed superior therapeutic outcomes as compared to cisplatin (which is a non-ICD-inducing platinum agent) plus ICI (clinical trials CheckMate 649 vs Keynote 062). Thus, in patients with advanced unresectable gastric cancer or gastro-esophageal junction (GEJ) carcinomas, oxaliplatin but not cisplatin induced synergistic effects with PD-1 ICI leading to improved PFS and OS as compared to chemotherapy alone (52). Similarly, patients with HER-2⁺ GEJ carcinomas that received oxaliplatin-based chemotherapy plus HER2 blocking monoclonal antibody in combination with PD-1 ICI exhibited an improved ORR of 24.3% as compared to 11.8% in patients receiving cisplatin instead of oxaliplatin (77). In sum, it has become clear that ICD-mediated preconditioning sets the stage for efficient PD-1/PD-L1 ICI in a variety of solid tumors. For this reason, current ICI treatment regimens in clinical routine should be optimized by preconditioning of the tumor immune microenvironment with immunogenic chemotherapy. Nevertheless, despite overwhelming evidence for the superior efficacy of ICD-inducing oxaliplatin-based combination therapies with ICI in lung and gastric cancer, the distinct immunogenic properties of platinum-based agents are still not fully appreciated by regulatory instances. Oncological guidelines need to be updated with recommendations to prioritize the use of immunogenic platinum compounds for subsequent curative ICI.

Data availability statement: All data sources are cited in this manuscript.

Funding

O.K. received funding from the Institut National du Cancer (INCa) and the DIM ELICIT initiative of the Ile de France. GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; European Research Council Advanced Investigator Grant “ICD-Cancer”, Fondation pour la Recherche Médicale (FRM); a donation by Elior; Equipex Onco-Pheno-Screen; European Joint Programme on Rare Diseases (EJPRD); European Research Council (ICD-Cancer), European Union Horizon 2020 Projects Oncobiome and Crimson; Fondation Carrefour; Institut National du Cancer (INCa); Institut Universitaire de France; LabEx Immuno-Oncology (ANR-18-IDEX-0001); a Cancer Research ASPIRE Award from the Mark Foundation; the RHU Immunolife; Seerave Foundation; SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and SIRIC Cancer Research and Personalized Medicine (CARPEM). This study contributes to the IdEx Université de Paris ANR-18-IDEX-0001. H.P. is supported by the Chinese scholarship council.

Conflicts of interest

O.K is a scientific co-founder of Samsara Therapeutics. Outside of this work, GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, and Vascage. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific

co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is in the scientific advisory boards of Hevolution, Institut Servier and Longevity Vision Funds. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. GK's wife, Laurence Zitvogel, has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9 m, Tusk and Roche, was on the on the Board of Directors of Transgene, is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim.

References

Figure Legends

Figure 1. ICI targeting the PD-1/PD-L1 axis. T cell receptor (TCR) expressed on T lymphocytes can engage with tumor antigen presented by malignant cells via histocompatibility complex II (MHC II) leading to the formation of an immunological synapse and the activation of effector cells. Parallel expression of PD-L1 on cancer cells and binding to PD-1 receptors on T cells can lead to premature exhaustion thus negatively regulating T cell functions. Monoclonal antibodies designed to target PD-1 or PD-L1 can sterically inhibit their interaction thus operating as immune checkpoint inhibitors.

Figure 2. Immunogenic cell death-mediated TME preconditioning. Induction of immunogenic cell stress and death (ICD) can trigger the emission of danger associated molecular patterns (DAMP) by malignant cells in turn leading to the attraction and activation of antigen presenting cells. Tumor antigen presentation to T cells and the resulting priming of cytotoxic T lymphocytes (CTL) further stimulates the infiltration of the tumor microenvironment (TME) by immune cells. Altogether ICD induction facilitates immune infiltration thus converting an immune scarce (cold) into an immune rich (hot) TME.

Figure 3. Sequential ICD+ICI combination therapy. Multiple lines of evidence support the idea that the sequential combination of immunogenic cell death (ICD) induction, which triggers the infiltration of the tumor microenvironment by adaptive immune effector cells, with subsequent immune checkpoint blockade, which inhibits premature T cell exhaustion, triggers therapeutically important synergistic effects.

1. Goldstein D, Laszlo J. The role of interferon in cancer therapy: a current perspective. *CA Cancer J Clin.* 1988;38(5):258-77.
2. Jiang T, Zhou C, Ren S. Role of IL-2 in cancer immunotherapy. *Oncoimmunology.* 2016;5(6):e1163462.
3. Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer--a current perspective. *Nat Rev Urol.* 2014;11(3):153-62.
4. Kroemer G, Galassi C, Zitvogel L, Galluzzi L. Immunogenic cell stress and death. *Nat Immunol.* 2022;23(4):487-500.
5. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010;363(8):711-23.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366(26):2443-54.
7. Byun DJ, Wolchok JD, Rosenberg LM, Girotra M. Cancer immunotherapy - immune checkpoint blockade and associated endocrinopathies. *Nat Rev Endocrinol.* 2017;13(4):195-207.
8. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T cell activation. *J Exp Med.* 1996;183(6):2541-50.
9. Chambers CA, Allison JP. Co-stimulation in T cell responses. *Curr Opin Immunol.* 1997;9(3):396-404.
10. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA. CTLA-4 can function as a negative regulator of T cell activation. *Immunity.* 1994;1(5):405-13.
11. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med.* 2000;192(7):1027-34.
12. Ledford H, Else H, Warren M. Cancer immunologists scoop medicine Nobel prize. *Nature.* 2018;562(7725):20-1.
13. Korman AJ, Garrett-Thomson SC, Lonberg N. The foundations of immune checkpoint blockade and the ipilimumab approval decennial. *Nat Rev Drug Discov.* 2022;21(7):509-28.
14. Johnson DB, Nebhan CA, Moslehi JJ, Balko JM. Immune-checkpoint inhibitors: long-term implications of toxicity. *Nat Rev Clin Oncol.* 2022;19(4):254-67.
15. Sondak VK, Smalley KS, Kudchadkar R, Gripton S, Kirkpatrick P. Ipilimumab. *Nat Rev Drug Discov.* 2011;10(6):411-2.
16. Heinzerling L, Ott PA, Hodi FS, Husain AN, Tajmir-Riahi A, Tawbi H, Pauschinger M, Gajewski TF, Lipson EJ, Luke JJ. Cardiotoxicity associated with CTLA4 and PD1 blocking immunotherapy. *J Immunother Cancer.* 2016;4:50.
17. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med.* 2015;373(1):23-34.

18. Haslam A, Gill J, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for Immune Checkpoint Inhibitor Drugs. *JAMA Netw Open*. 2020;3(3):e200423.
19. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med*. 2007;13(1):54-61.
20. Schiavoni G, Sistigu A, Valentini M, Mattei F, Sestili P, Spadaro F, Sanchez M, Lorenzi S, D'Urso MT, Belardelli F, et al. Cyclophosphamide synergizes with type I interferons through systemic dendritic cell reactivation and induction of immunogenic tumor apoptosis. *Cancer Res*. 2011;71(3):768-78.
21. Yamazaki T, Buque A, Ames TD, Galluzzi L. PT-112 induces immunogenic cell death and synergizes with immune checkpoint blockers in mouse tumor models. *Oncoimmunology*. 2020;9(1):1721810.
22. Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, Aymeric L, Michaud M, Apetoh L, Barault L, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene*. 2010;29(4):482-91.
23. Obeid M, Panaretakis T, Joza N, Tufi R, Tesniere A, van Endert P, Zitvogel L, Kroemer G. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. *Cell Death Differ*. 2007;14(10):1848-50.
24. Petrazzuolo A, Perez-Lanzon M, Martins I, Liu P, Kepp O, Minard-Colin V, Maiuri MC, Kroemer G. Pharmacological inhibitors of anaplastic lymphoma kinase (ALK) induce immunogenic cell death through on-target effects. *Cell Death Dis*. 2021;12(8):713.
25. Spisek R, Charalambous A, Mazumder A, Vesole DH, Jagannath S, Dhodapkar MV. Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. *Blood*. 2007;109(11):4839-45.
26. Gomes-da-Silva LC, Zhao L, Bezu L, Zhou H, Sauvat A, Liu P, Durand S, Leduc M, Souquere S, Loos F, et al. Photodynamic therapy with redaporfin targets the endoplasmic reticulum and Golgi apparatus. *EMBO J*. 2018;37(13).
27. Vitale I, Senovilla L, Jemaa M, Michaud M, Galluzzi L, Kepp O, Nanty L, Criollo A, Rello-Varona S, Manic G, et al. Multipolar mitosis of tetraploid cells: inhibition by p53 and dependency on Mos. *EMBO J*. 2010;29(7):1272-84.
28. Galluzzi L, Vacchelli E, Fridman WH, Galon J, Sautes-Fridman C, Tartour E, Zucman-Rossi J, Zitvogel L, Kroemer G. Trial Watch: Monoclonal antibodies in cancer therapy. *Oncoimmunology*. 2012;1(1):28-37.
29. Petrazzuolo A, Perez-Lanzon M, Liu P, Maiuri MC, Kroemer G. Crizotinib and ceritinib trigger immunogenic cell death via on-target effects. *Oncoimmunology*. 2021;10(1):1973197.
30. Zitvogel L, Perreault C, Finn OJ, Kroemer G. Beneficial autoimmunity improves cancer prognosis. *Nat Rev Clin Oncol*. 2021;18(9):591-602.
31. Katayama H, Kobayashi M, Irajizad E, Sevillano A, Patel N, Mao X, Rusling L, Vykoukal J, Cai Y, Hsiao F, et al. Protein citrullination as a source of cancer neoantigens. *J Immunother Cancer*. 2021;9(6).
32. Zhou Y, Bastian IN, Long MD, Dow M, Li W, Liu T, Ngu RK, Antonucci L, Huang JY, Phung QT, et al. Activation of NF-kappaB and p300/CBP potentiates cancer

chemoimmunotherapy through induction of MHC-I antigen presentation. *Proc Natl Acad Sci U S A*. 2021;118(8).

33. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer*. 2012;12(12):860-75.
34. Lotze MT, Deisseroth A, Rubartelli A. Damage associated molecular pattern molecules. *Clin Immunol*. 2007;124(1):1-4.
35. Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol*. 2007;28(10):429-36.
36. Matzinger P. An innate sense of danger. *Ann N Y Acad Sci*. 2002;961:341-2.
37. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002;296(5566):301-5.
38. Fucikova J, Moserova I, Urbanova L, Bezu L, Kepp O, Cremer I, Salek C, Strnad P, Kroemer G, Galluzzi L, et al. Prognostic and Predictive Value of DAMPs and DAMP-Associated Processes in Cancer. *Front Immunol*. 2015;6:402.
39. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol*. 2020;20(2):95-112.
40. Kepp O, Bezu L, Yamazaki T, Di Virgilio F, Smyth MJ, Kroemer G, Galluzzi L. ATP and cancer immunosurveillance. *EMBO J*. 2021;40(13):e108130.
41. Vacchelli E, Ma Y, Baracco EE, Sistigu A, Enot DP, Pietrocola F, Yang H, Adjemian S, Chaba K, Semeraro M, et al. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. *Science*. 2015;350(6263):972-8.
42. Obeid M, Panaretakis T, Tesniere A, Joza N, Tufi R, Apetoh L, Ghiringhelli F, Zitvogel L, Kroemer G. Leveraging the immune system during chemotherapy: moving calreticulin to the cell surface converts apoptotic death from "silent" to immunogenic. *Cancer Res*. 2007;67(17):7941-4.
43. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, Bratton DL, Oldenborg PA, Michalak M, Henson PM. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell*. 2005;123(2):321-34.
44. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remedios C, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat Med*. 2014;20(11):1301-9.
45. Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, Fujioka Y, Ohba Y, Gorman JV, Colgan JD, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. *Nat Immunol*. 2012;13(9):832-42.
46. Vitale I, Shema E, Loi S, Galluzzi L. Intratumoral heterogeneity in cancer progression and response to immunotherapy. *Nat Med*. 2021;27(2):212-24.
47. Galluzzi L, Humeau J, Buque A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol*. 2020;17(12):725-41.
48. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313(5795):1960-4.

49. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*. 2020;20(11):662-80.
50. Kepp O, Marabelle A, Zitvogel L, Kroemer G. Oncolysis without viruses - inducing systemic anticancer immune responses with local therapies. *Nat Rev Clin Oncol*. 2020;17(1):49-64.
51. Yamazaki K, Nagase M, Tamagawa H, Ueda S, Tamura T, Murata K, Eguchi Nakajima T, Baba E, Tsuda M, Moriwaki T, et al. Randomized phase III study of bevacizumab plus FOLFIRI and bevacizumab plus mFOLFOX6 as first-line treatment for patients with metastatic colorectal cancer (WJOG4407G). *Ann Oncol*. 2016;27(8):1539-46.
52. Liu P, Chen J, Zhao L, Hollebecque A, Kepp O, Zitvogel L, Kroemer G. PD-1 blockade synergizes with oxaliplatin-based, but not cisplatin-based, chemotherapy of gastric cancer. *Oncoimmunology*. 2022;11(1):2093518.
53. Zhou H, Tu C, Yang P, Li J, Kepp O, Li H, Zhang L, Zhang L, Zhao Y, Zhang T, et al. Carbon ion radiotherapy triggers immunogenic cell death and sensitizes melanoma to anti-PD-1 therapy in mice. *Oncoimmunology*. 2022;11(1):2057892.
54. Kroemer G, Kepp O. Small cell lung cancer responds to immunogenic chemotherapy followed by PD-1 blockade. *Oncoimmunology*. 2021;10(1):1996686.
55. Levesque S, Le Naour J, Pietrocola F, Paillet J, Kremer M, Castoldi F, Baracco EE, Wang Y, Vacchelli E, Stoll G, et al. A synergistic triad of chemotherapy, immune checkpoint inhibitors, and caloric restriction mimetics eradicates tumors in mice. *Oncoimmunology*. 2019;8(11):e1657375.
56. Xin M, Lin D, Yan N, Li H, Li J, Huang Z. Oxaliplatin facilitates tumor-infiltration of T cells and natural-killer cells for enhanced tumor immunotherapy in lung cancer model. *Anticancer Drugs*. 2022;33(2):117-23.
57. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, Yamazaki T, Poirier-Colame V, Newton A, Redouane Y, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. *Immunity*. 2016;44(2):343-54.
58. Sun F, Cui L, Li T, Chen S, Song J, Li D. Oxaliplatin induces immunogenic cells death and enhances therapeutic efficacy of checkpoint inhibitor in a model of murine lung carcinoma. *J Recept Signal Transduct Res*. 2019;39(3):208-14.
59. Song W, Shen L, Wang Y, Liu Q, Goodwin TJ, Li J, Dorosheva O, Liu T, Liu R, Huang L. Synergistic and low adverse effect cancer immunotherapy by immunogenic chemotherapy and locally expressed PD-L1 trap. *Nat Commun*. 2018;9(1):2237.
60. Wang W, Wu L, Zhang J, Wu H, Han E, Guo Q. Chemoimmunotherapy by combining oxaliplatin with immune checkpoint blockades reduced tumor burden in colorectal cancer animal model. *Biochem Biophys Res Commun*. 2017;487(1):1-7.
61. Maharjan R, Choi JU, Kweon S, Pangeni R, Lee NK, Park SJ, Chang KY, Park JW, Byun Y. A novel oral metronomic chemotherapy provokes tumor specific immunity resulting in colon cancer eradication in combination with anti-PD-1 therapy. *Biomaterials*. 2022;281:121334.
62. Limagne E, Thibaudin M, Nuttin L, Spill A, Derangere V, Fumet JD, Amellal N, Peranzoni E, Cattani V, Ghiringhelli F. Trifluridine/Tipiracil plus Oxaliplatin Improves PD-1 Blockade in Colorectal Cancer by Inducing Immunogenic Cell Death and Depleting Macrophages. *Cancer Immunol Res*. 2019;7(12):1958-69.

63. Grasselly C, Denis M, Bourguignon A, Talhi N, Mathe D, Tourette A, Serre L, Jordheim LP, Matera EL, Dumontet C. The Antitumor Activity of Combinations of Cytotoxic Chemotherapy and Immune Checkpoint Inhibitors Is Model-Dependent. *Front Immunol.* 2018;9:2100.
64. Kim W, Chu TH, Nienhuser H, Jiang Z, Del Portillo A, Remotti HE, White RA, Hayakawa Y, Tomita H, Fox JG, et al. PD-1 Signaling Promotes Tumor-Infiltrating Myeloid-Derived Suppressor Cells and Gastric Tumorigenesis in Mice. *Gastroenterology.* 2021;160(3):781-96.
65. Zhu H, Shan Y, Ge K, Lu J, Kong W, Jia C. Oxaliplatin induces immunogenic cell death in hepatocellular carcinoma cells and synergizes with immune checkpoint blockade therapy. *Cell Oncol (Dordr).* 2020;43(6):1203-14.
66. Liu P, Zhao L, Pol J, Levesque S, Petrazzuolo A, Pfirschke C, Engblom C, Rickelt S, Yamazaki T, Iribarren K, et al. Crizotinib-induced immunogenic cell death in non-small cell lung cancer. *Nat Commun.* 2019;10(1):1486.
67. Liu P, Zhao L, Kepp O, Kroemer G. Crizotinib - a tyrosine kinase inhibitor that stimulates immunogenic cell death. *Oncoimmunology.* 2019;8(7):1596652.
68. Siew YY, Neo SY, Yew HC, Lim SW, Ng YC, Lew SM, Seetoh WG, Seow SV, Koh HL. Oxaliplatin regulates expression of stress ligands in ovarian cancer cells and modulates their susceptibility to natural killer cell-mediated cytotoxicity. *Int Immunol.* 2015;27(12):621-32.
69. Xie W, Mondragon L, Mauseth B, Wang Y, Pol J, Levesque S, Zhou H, Yamazaki T, Eksteen JJ, Zitvogel L, et al. Tumor lysis with LTX-401 creates anticancer immunity. *Oncoimmunology.* 2019;8(7):1594555.
70. D'Amico L, Menzel U, Prummer M, Muller P, Buchi M, Kashyap A, Haessler U, Yermanos A, Gebleux R, Briendl M, et al. A novel anti-HER2 anthracycline-based antibody-drug conjugate induces adaptive anti-tumor immunity and potentiates PD-1 blockade in breast cancer. *J Immunother Cancer.* 2019;7(1):16.
71. Xu J, Xu N, Bai Y, Liu R, Mao C, Sui H, Wang X, Jiang Q, Dou Y. Anti-PD-1 antibody HX008 combined with oxaliplatin plus capecitabine for advanced gastric or esophagogastric junction cancer: a multicenter, single-arm, open-label, phase Ib trial. *Oncoimmunology.* 2020;10(1):1864908.
72. Kepp O, Zitvogel L, Kroemer G. Clinical evidence that immunogenic cell death sensitizes to PD-1/PD-L1 blockade. *Oncoimmunology.* 2019;8(10):e1637188.
73. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Kurata T, Chiappori A, Lee KH, de Wit M, et al. Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. *N Engl J Med.* 2018;379(24):2342-50.
74. Faivre-Finn C, Vicente D, Kurata T, Planchard D, Paz-Ares L, Vansteenkiste JF, Spigel DR, Garassino MC, Reck M, Senan S, et al. Four-Year Survival With Durvalumab After Chemoradiotherapy in Stage III NSCLC-an Update From the PACIFIC Trial. *J Thorac Oncol.* 2021;16(5):860-7.
75. Voorwerk L, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, Nederlof I, Kluin RJC, Warren S, Ong S, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat Med.* 2019;25(6):920-8.
76. Akamatsu H, Teraoka S, Hayashi H, Fujimoto D, Hayata A, Haratani K, Ozawa Y, Yoshida T, Iwasa T, Shimokawa T, et al. Pembrolizumab Plus Amrubicin in Patients With

Relapsed SCLC: Multi-Institutional, Single-Arm Phase 2 Study. *JTO Clin Res Rep.* 2021;2(7):100184.

77. Janjigian YY, Kawazoe A, Yanez P, Li N, Lonardi S, Kolesnik O, Barajas O, Bai Y, Shen L, Tang Y, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. *Nature.* 2021;600(7890):727-30.

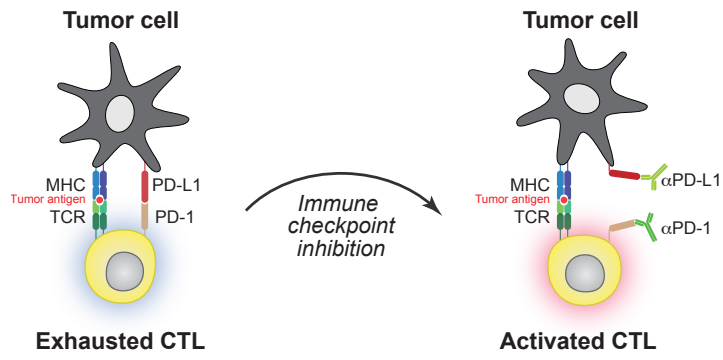


Figure 1

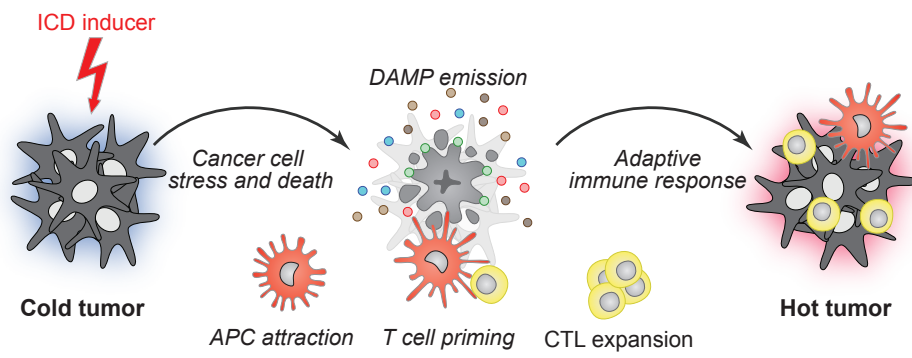


Figure 2

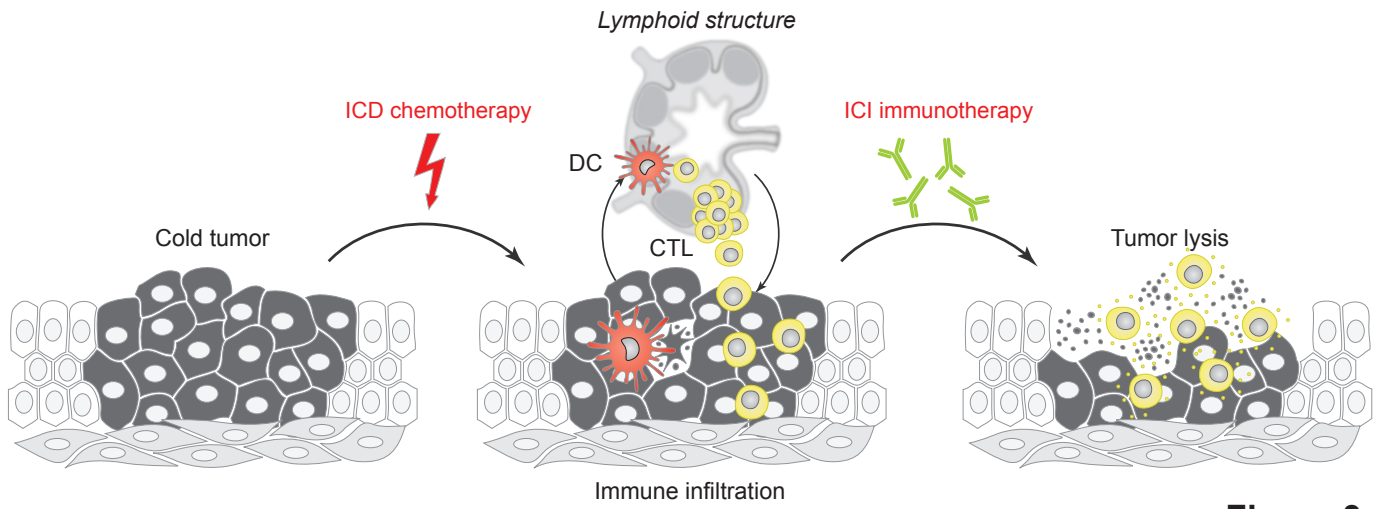


Figure 3