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► To cite this version:

Peng Liu, Liwei Zhao, Laurence Zitvogel, Oliver Kepp, Guido Kroemer. The BCL2 inhibitor venetoclax mediates anticancer effects through dendritic cell activation. *Cell Death and Differentiation*, 2023, 30 (12), pp.2447-2451. 10.1038/s41418-023-01232-y . hal-04601419

HAL Id: hal-04601419

<https://hal.science/hal-04601419v1>

Submitted on 4 Jun 2024

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The BCL2 inhibitor venetoclax mediates anticancer effects through dendritic cell activation

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Abstract

BCL2 is an apoptosis-inhibitory oncoprotein that also possesses apoptosis-unrelated activities. Pharmacological BCL2 inhibitors have been developed with the scope of driving BCL2-dependent cancer cells into apoptosis, and one BCL2 antagonist, venetoclax, has been clinically approved for the treatment of specific leukemias and lymphomas. Nonetheless, it appears that venetoclax, as well as genetic BCL2 inhibition, can mediate anticancer effects through an indirect action. Such an indirect effect relies on the enhancement of the immunostimulatory function of dendritic cells, hence increasing tumor immunosurveillance. Mechanistically, BCL2 inhibition involves improved antigen presentation by conventional type-1 dendritic cells (cDC1s) due to the activation of an interferon response, leading to a T cell-mediated anticancer immune response that can be further enhanced by PD-1 blockade. These findings support the emerging hypothesis that successful antineoplastic drugs generally mediate their effects indirectly, through the immune system, rather via merely cell-autonomous effects on malignant cells.

Facts

- BCL2 inhibitors suppress cancer growth in an immunocompetent but not in an immunodeficient context.
- BCL2 inhibition improves the capacity of cDC1 to present tumor antigen and to stimulate anticancer immunity.
- BCL2 blockade sensitizes tumors to PD-1/PD-L1-immune checkpoint blockade.

Open Questions

- How does BCL2 inhibition exert cDC1 activation?
- In what way can BCL2 inhibitors be used for improving standard immunotherapy?

Main text

B-cell lymphoma-2 (BCL2) has been initially discovered as an oncogene/oncoprotein that is overexpressed in follicular lymphoma^{1,2,3}. BCL2 was later found to inhibit apoptosis through its capacity to inhibit mitochondrial permeabilization leading to the release of caspase activators and caspase-independent death effectors^{4,5,6}. This mitochondrion-sealing effect of BCL2 has been explained by its action on other proteins of an extended family of proteins possessing the so called BCL2 homology (BH1, BH2, BH3, BH4) domains, which together form the BCL2 family of proteins^{7,8}. In particular, BCL2 was shown to directly interact with other proteins from the BCL2 family, to inhibit pore formation by pro-apoptotic proteins such as BAX and BAK (which both possess BH1, BH2 and BH3 but not BH4 domains) and to intercept (some of) the pro-apoptotic BH3-only proteins as well^{9,10}. For long, it was believed that the oncogenic function of BCL2 could be completely ascribed to its apoptosis-inhibitory action. Nonetheless, BCL2 has been found to mediate additional, apoptosis-independent cellular effects, including the inhibition of autophagy, inflammation and senescence, as well as broad effects on cellular Ca²⁺ fluxes, redox homeostasis and bioenergetic metabolism^{11,12,13}.

Small molecule inhibitors of BCL2 have been generated by the pharmacological industry^{14,15} and one of them, venetoclax, has been approved for clinical use against BCL2 dependent cancers, in particular chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL)^{16,17,18,19,20}. Moreover, venetoclax can be combined with other drugs (such as azacitidine, decitabine or cytarabine) for the treatment of acute myeloid leukemia (AML)²¹. Since these effects reportedly are mediated by direct killing of cancer cells, it has been assumed that the apoptosis-unrelated effects of BCL2 are not relevant for the anticancer effects of BCL2 inhibitors.

Recently, we performed a genome-wide screen to identify genes the knockout of which can enhance antigen presentation by dendritic cells (DCs)²². DCs are terminally differentiated cells, meaning that genetic screens on such cells are impractical²³. To circumvent this difficulty, we resorted to an immortalized DC precursor cell lines that expresses the SV40 large-T-cell antigen to inactivate the tumor suppressors TP53 and RB under the control of a doxycycline-inducible promoter^{24, 25}. If cultured in the presence of doxycycline and the synthetic glucocorticoid dexamethasone, these immortalized DC precursor cell lines can be cultured indefinitely^{24, 25, 26, 27}, allowing us to stably introduce the CRISP/Cas9 system into the cells and to transfect the cells with a genome-wide collection of barcoded guidance RNAs²². Upon removal of doxycycline and dexamethasone, the DC precursors lose SV40 expression and differentiate into mature DCs that resemble one particular DC subset, namely conventional type 1 DCs (cDC1s) at the level of the transcriptome. Moreover, the de-immortalized mature DCs generated in vitro resemble cDC1s with respect to the capacity to sample proteins present in the extracellular fluid, giving them access to the cytosol and to the antigen-presenting machinery. Indeed, such cDC1s are the only cells in the body that, in the presence of extracellular cytochrome *c*, undergo apoptosis because cytochrome *c* freely enters the cytosol where it activates apoptosomes containing APAF1 and caspase-9²⁸. We therefore refer to our artificial DCs as cDC1s.

Based on our genome-wide screen performed on cDC1s, we identified BCL2 as a gene the knockout of which confers a gain-of-function phenotype²². This gain-of-function phenotype could be mimicked by using pharmacological BCL2 inhibitors including the clinically approved drug venetoclax and the experimental drug navitoclax. Importantly, genetic or pharmacological inhibition of BCL2 did not compromise the survival of cDC1s in vitro or in

vivo, suggesting that enhanced antigen presentation occurs independently from the apoptotic pathway²².

DCs, in particular of the cDC1 type, play a decisive role in the initiation and stimulation of cellular immune responses mediated by T lymphocytes^{29, 30}. Based on this knowledge, we wondered whether BCL2 inhibition might have a general immunostimulatory effect that might improve cancer immunosurveillance. In the course of this experimental exploration, we obtained multiple results pleading in favor of the idea that BCL2 inhibition can exert antineoplastic effects through an action on cDC1s (**Fig. 1**).

In vitro experiments on murine cancer cell lines. When added to cutaneous fibrosarcoma MCA205 cells or non-small cell lung cancer (NSCLC) TC1 cells, the BCL2 inhibitors navitoclax and venetoclax largely fail to induce apoptosis. This contrasts with the fact that such BCL2 inhibitors can be administered to immunocompetent mice bearing orthotopic MCA205 or TC1 tumors under the skin or in the lung, respectively, and then cause a decrease in tumor growth. This anticancer effect is completely lost when T lymphocytes are depleted by antibodies recognizing CD4 and CD8, demonstrating that they require a cellular immune response²². A similar result has been previously described for MC38 colorectal cancer cells that can form subcutaneous tumors if inoculated into mice. Such ectopic cancers also respond to venetoclax only in immunocompetent, not in T cell-deficient, mice³¹. Moreover, MC38 cells do not undergo apoptosis when cultured in the presence of venetoclax³¹. Hence, cancers that do not respond to BCL2 inhibitors in vitro can respond to such inhibitors in vivo, but only in the context of an intact immune system.

In vitro experiments on cDC1s. Knockout of BCL2 in cDC1s improves their antigen-presenting function in vitro coupled to the upregulation of multiple co-stimulatory signals (such as CD80 and CD86), cytokines (such as IL-12), as well as a massive Type-1 interferon response²². Pharmacological inhibition of BCL2 with navitoclax or venetoclax in cDC1s phenocopies the in vitro effects of the BCL2 knockout, but does not further enhance the antigen-presenting function of BCL2-deficient cDC1s, demonstrating that this is an on-target effect. Improved antigen presentation by BCL2-inhibited cDC1 is lost upon neutralization of CD80, CD86 and the common Type-1 interferon receptor (IFNAR), unraveling the mechanisms of immunostimulation²².

In vivo effects of BCL2 inhibitors on cDC1s. The intravenous administration of navitoclax or venetoclax to mice results in the activation of cDC1s (but less so cDC2s) with an increased expression of the costimulatory molecules CD80 and CD86, the chemokine receptors CCR2, CCR7 and XCR1, as well as the activation marker MHC class-II. High-dimensional immunofluorescence cytometry confirmed that, in patients with AML treated with venetoclax, CCR2, CCR7, XCR1, CD5, CD86 and HLA-DQ were upregulated in circulating cDC1s (not cDC2s). Similar effects were obtained ex vivo when peripheral blood mononuclear cells from health donors were treated with venetoclax in vitro and the phenotypic properties of cDC1s were then analyzed by immunofluorescence cytometry²². Hence, pharmacological inhibition of BCL2 results in the activation of cDC1s (not cDC2s) in both human and mouse.

In vivo effects of BCL2-inhibited cDC1s. Intravenous injection of cDC1s that have been primed with tumor antigens in vitro confers some antitumor activity in vivo, in tumor-bearing immunocompetent mice, but this activity is enhanced when BCL2 knockout cDC1s are used or when WT cDC1s are pretreated with navitoclax or venetoclax prior to their injection²².

These effects of intravenously injected BCL2-inhibited cDC1s are particularly striking in BATF3 knockout cells (which lack the cDC1 subset and hence exhibit a major defect in immunosurveillance)³² but are lost in mice lacking mature T cells²². Of note, BCL2 knockout cDC1 acquire the transcriptional phenotype of migratory cDC1s (a phenotype that is not seen in WT cDC1s) and infiltrate lung cancer lesions more efficiently than WT cDC1s²². Hence, BCL2-inhibited cDC1s acquire a gain-of-function phenotype that improves their capacity to ignite cellular immune responses against tumor-associated antigens.

In vivo effects of BCL2 inhibitors on cancer immunosurveillance. Injection of navitoclax or venetoclax into immunocompetent mice slows down the progression, or causes the regression, of tumors that do not respond to navitoclax or venetoclax in vitro, especially if combined with PD-1 blockade²². This in vivo anticancer activity of navitoclax or venetoclax is lost when the cellular immune system can be compromised due to (i) knockout of the essential cDC1 lineage-specific transcription factor BATF3 causing the constitutive absence of cDC1s³²; (ii) repeated intravenous injection of cytochrome *c* for the acquired depletion of cDC1s²⁸; (iii) antibody-mediated blockade of CD11b, which prevents cDC1s to enter tumor beds or tumor-draining lymph nodes³³; (iv) antibody-mediated neutralization of IFNAR, which is the common Type-1 interferon receptor; and (v) depletion of T cells by injection of suitable anti-CD4 and anti-CD8 antibodies²². Of note, it appears that the effects of venetoclax on T cells occur downstream of the effects on cDC1s. Thus, venetoclax upregulates the expression of PD-1 and CTLA-4 on CD4⁺ and CD8⁺ T cells in vivo, in tumor-bearing mice, but this effect is lost upon depletion of cDC1s by cytochrome *c* injections²².

Altogether, the aforementioned results (**Fig. 1**) suggest that BCL2 inhibition can be active against cancers that are not BCL2-dependent (and hence do not undergo apoptosis upon BCL2

knockout or neutralization) through an indirect effect via the immune system that is primarily involving the activation of cDC1s ²². Mechanistically, it appears that genetic or pharmacological inhibition of BCL2 causes the release of DNA from mitochondria in cDC1s, thereby activating the cGAS/STING pathway, leading to the initially cell-autonomous activation of a classical Type-1 interferon pathway. Through autocrine or paracrine effects of Type-1 interferons on IFNAR, cDC1s then undergo transcriptional and functional reprogramming and improve their capacity to migrate into tumors, to present tumor antigens to T cells and to activate them. T cell activation is coupled to the upregulation of PD-1 (and CTLA-4) and specific T cell subsets. As an end result of this cascade of events instigated by BCL2 inhibition, tumor antigen-specific T cells infiltrate the cancer. This improves immunosurveillance and sensitizes the host to immune checkpoint inhibition (ICI) targeting the interaction between PD-1 and PD-L1 (**Fig. 2**).

Altogether, these findings add to the general concept that anticancer drugs generally do not (only) act on malignant cells, in spite of the fact that they have been conceived to kill them as cytotoxic chemotherapeutics or ‘targeted’ agents interfering with specific survival pathways ³⁴, ³⁵. Rather, successful anticancer drugs have empirically been selected by clinicians because of their capacity of inducing antitumor immune responses that account for treatment responses ³⁶, ³⁷. The major question that emerges from these considerations concerns the mechanisms of venetoclax against AML, CML and SLL. Does venetoclax favor anticancer immune responses against leukemia and lymphoma cells that might explain its true mode action, well beyond its cell-autonomous pro-apoptotic effects? Although there is no direct evidence in favor of this hypothesis, it appears intriguing that patients with AML can respond to immunotherapy with interleukin-2 ³⁸, vaccination with AML cell fused with autologous dendritic cells ³⁹, the PD-1 blocking antibody nivolumab ⁴⁰ or bispecific T cell engager antibodies ⁴¹. Hence, AML cells

are potentially immunogenic. Moreover, the prognosis of AML patients treated with chemotherapy is particularly positive when AML blasts exhibit signs of immunogenicity (such as calreticulin exposure)^{42, 43} or are recognized by AML-specific antibodies detectable in the plasma⁴⁴. Thus, it is possible, yet remains to be formally demonstrated, that blood cancers including AML are under therapy-relevant immunosurveillance. If this conjecture is correct, it would be highly plausible that venetoclax-triggered cDC1 activation contributes to the therapeutic success of this BCL2 inhibitor.

Historically, cell death research has been focusing on cell-intrinsic pathway, hoping to identify cell-autonomous pathways that can be targeted for selectively killing cancer cells or preserving normal cells. The senior author of this paper has participated to these bygone - and largely vain - efforts that were based on an over-simplistic view of cell (death) biology. Indeed, in vivo, cell death never occurs in isolation, as this might happen in the artificial setting of cell cultures, but rather within the context of a complex multicellular ecosystem. Such an ecosystem shapes the organismal response to cellular demise consisting in, for example, silent efferocytosis, inflammation and subsequent repair, as well as in innate or acquired immune responses against dead-cell antigens. Hence, the question whether and how cells die in isolation is superseded in importance by the question how cell stress and death are perceived by the organism^{45, 46, 47, 48, 49, 50}.

In this general context, it is not surprising that the clinical success of BCL2 inhibition finally is not (just) attributable to direct cancer cell killing but rather involves favorable effects on the cancer-immunity dialogue. In this vein, it will be interesting to determine whether venetoclax or other BCL2 inhibitors may be repurposed to stimulate anticancer immune responses against a vast spectrum of oncological diseases, likely in the context of immune checkpoint inhibitors

or other modalities of immunotherapy. Hence, the finding that BCL2 inhibitors likely mediate their therapeutic effect not (only) by killing BCL2-dependent malignant cells ultimately might yield a luminous perspective. Thanks to their immunostimulatory action on cDC1s, BCL2 inhibitors might mediate rather broad, target-agnostic anticancer effects. Beyond this consideration, it will be interesting to identify other pharmacological agents that, like BCL2 inhibitors, enhance the functional capabilities of cDC1s. Indeed, such drugs that enhance the function of cDC1s (to which we refer as “ICD enhancers”)⁵¹ might be used as a new class of immune checkpoint inhibitors to treat neoplastic disease.

Conflict of interest statement

OK and GK have been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Tollys, and Vascage. LZ 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 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. OK is a scientific co-founder of Samsara Therapeutics. 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 brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. The funders had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgments

OK receives funding from Institut National du Cancer (INCa); 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; Fondation pour la Recherche Médicale (FRM); a donation by Elior; Equipex Onco-Pheno-Screen; European Joint Programme on Rare Diseases (EJPRD); European Research Council Advanced Investigator Award (ERC-2021-ADG, ICD-Cancer, Grant No. 101052444), European Union Horizon 2020 Projects Oncobiome, Prevalung (grant No. 101095604) and Crimson; 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. Views and opinions expressed are those of the author(s) only and do not

necessarily reflect those of the European Union, the European Research Council or any other granting authority. Neither the European Union nor any other granting authority can be held responsible for them.

Author Contributions

PL and LZ summarized data and designed display items, LZ, OK and GK wrote the manuscript.

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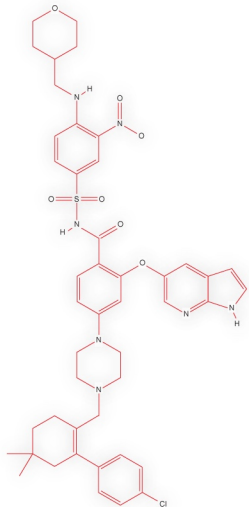
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Figure legends

Figure 1: Mechanisms of anticancer effects of venetoclax against solid cancers. For details consult main text. The chemical structure of venetoclax was adapted from Pubchem.

Figure 2: Cascade of molecular, cellular and immunological events linking the inhibition of BCL2 to the stimulation of anticancer immune responses in vivo. For details consult main text.



Venetoclax

- **No direct effects on most solid cancers**
(e.g. MC38, MCA205, TC1)
- **Activation of cDC1 in vitro, in mice and patients:**
 - Type-1 interferon response
 - Chemokine receptors + chemotaxis
 - Upregulation of MHC class II
 - Costimulation + antigen presentation
- **Anticancer effects in immunocompetent mice depending on:**
 - cDC1 subset of dendritic cells
Absent in *BATF3*^{-/-} mice
Depleted upon *i.v.* injection of cytochrome *c*
Extravasation blocked by α CD11b antibody
 - Type-1 interferon signaling
Blocked by antibody against IFNAR1
 - T cells (which upregulate PD-1 + CTLA-4 in a cDC1-dependent fashion)
Depleted by α CD4 and α CD8 antibodies
- **Anticancer effects enhanced by PD-1 blockade depending on:**
 - cDC1 subset of dendritic cells

Figure 1

cDC1

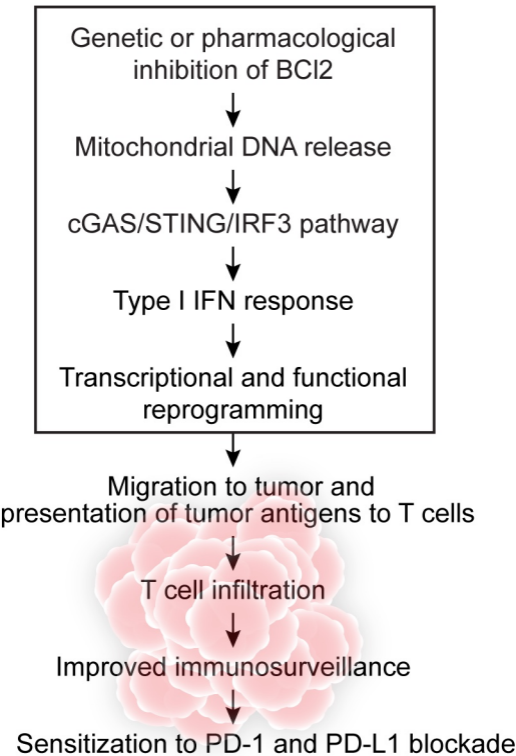


Figure 2