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Classification of chemotherapeutic agents based on their differential *in vitro* impacts on dendritic cells

Hiroaki Tanaka^{*,1}, Hironori Matsushima^{*,1,2}, Norikatsu Mizumoto¹, and Akira Takashima^{1,2}

¹Department of Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas

²Department of Medical Microbiology and Immunology, University of Toledo College of Medicine, Toledo, Ohio

Abstract

Despite the crucial roles dendritic cells (DCs) play in host immunity against cancer, pharmacological effects of many chemotherapeutic agents have remained mostly unknown. We recently developed the DC biosensor clone by engineering the stable murine DC line XS106 to express the yellow fluorescent protein (YFP) gene under the control of IL-1 β promoter. In this study, the resulting XS106 pIL1-YFP DC clone was used to screen 54 anticancer drugs. Each drug was tested at 5 concentrations (0.1-10 μ M) for the effects on YFP expression, cell viability, and GM-CSF-dependent growth. Our unbiased systematic screening unveiled striking heterogeneity among the tested anticancer drugs in their effects on the three functional parameters. Interestingly, 15 drugs induced significant YFP expression at sub-cytotoxic concentrations and were, thus, categorized as “DC-stimulatory” anticancer drugs. These drugs were subsequently found to induce at least one of the characteristic maturational changes in mouse bone marrow-derived DCs. For example, vinblastine a prototypic drug of this class induced production of IL-1 β , IL-6, and IL-12, elevated surface expression of CD40, CD80, CD86, and MHC class II, and an augmented T cell-stimulatory capacity of DCs. Not only do these results illustrate differential pharmacological effects of commonly used chemotherapeutic agents on DCs, they may also provide a conceptual framework for rationale-based selection and combination of anticancer drugs for clinical application.

Keywords

chemotherapy; pharmacology; dendritic cell

INTRODUCTION

DCs possess all functional properties required for presenting tumor-associated antigens to effector T cells and, thereby, protecting the host from tumor development (1). However, tumor cells may escape from immune surveillance and even shape their immunological phenotype *via* cancer immunoediting (2,3). For example, cancer cells (and tumor-infiltrating leukocytes) suppress the development/maturation of DCs by elaborating vascular endothelial growth factor, transforming growth factor- β , IL-10, and prostaglandin-E₂ (3,4). This problem may be overcome by DC-targeted immunotherapeutic approaches, such as adoptive transfer of *ex vivo* expanded DCs after loading with tumor antigens, intra-tumor administration of DC-

Address correspondence to: Akira Takashima, Department of Medical Microbiology and Immunology, University of Toledo College of Medicine, 3000 Arlington Ave., Toledo, OH 43614. Phone: 419-383-5423; Fax: 419-383-3002; E-mail: akira.takashima@UToledo.edu.

*These authors contributed equally to this work.

attracting chemokines, and use of DC-stimulatory adjuvants (5,6). Tissue-resident DCs play immunosurveillance roles in the steady state by sampling materials and detecting aberrant signals, including microbial products and pro-inflammatory mediators. Upon sensing such pathological signals, DCs elevate surface expression of MHC II and co-stimulatory molecules, elaborate cytokines and chemokines, heighten the ability to activate immunological naïve T cells, and migrate to draining lymph nodes. Those DCs that have completed these changes (termed mature DCs) are fully capable of inducing adaptive immune responses, whereas immature DCs are involved in immunological tolerance (7).

Chemotherapy remains the standard treatment modality for many advanced cancers, although it is neither curative as a stand-alone protocol nor effective in augmenting host immune responses to cancer cells. Instead, most chemotherapeutic agents are likely to impair clonal expansion of effector lymphocytes as well as homeostasis of innate leukocytes, thereby potentially suppressing host immunity (8). This is not totally unexpected because classic chemotherapeutic agents were originally discovered based on their activities to interrupt with metabolic processes for DNA, RNA, and protein biosynthesis. It has become evident recently that certain chemotherapeutics augment host immunity (9). For example, selected agents increase immunogenicity of cancer cells (10,11), preferentially inhibit the function of regulatory T cells (12,13), or elicit macrophage activation (14). Few systematic studies have been reported in the literature comparing diverse anticancer agents for their impacts on DCs. Thus, we sought to fill this apparent gap of knowledge by conducting unbiased screen of a broad spectrum of chemotherapeutic agents. To achieve this time- and cost-efficiently, we employed the recently developed DC biosensor system. Based on the observation that DC maturation is accompanied with rapid and robust IL-1 β mRNA expression (15), we engineered the murine DC line XS106 to express the YFP gene under the control of IL-1 β promoter. The resulting XS106-pIL1-YFP DC clone exhibited strong YFP fluorescent signals upon exposure to all tested agents known to induce DC maturation (15). Here we report differential impacts of 54 anticancer drugs on DC maturation, survival, and growth.

MATERIALS AND METHODS

Cell lines

The XS106-pIL1-YFP DC clone (15) and the parental XS106 DC line were maintained in the complete RPMI 1640 supplemented with 0.5 ng/ml GM-CSF and 5% NS cell-conditioned medium (16).

Reagents

Anticancer drugs with diverse chemical structures and mechanisms of action (17) were purchased from commercial vendors (Supplemental Table S1). All the drugs were dissolved in DMSO at 2 mM and tested at different concentrations with a final DMSO concentration of 0.5%. Ovalbumin (OVA) (Sigma) was dissolved in PBS at 100 mg/ml and then passed through the polymyxin B column (PIERCE) repeatedly until endotoxin became undetectable by the QCL-1000 system (Cambrex Bio Science).

Classification of anticancer drugs

The DC biosensor clone was incubated in 96 well plates (5×10^4 cells/200 μ l/well) for 16 h with each drug at five concentrations in triplicates and then examined for YFP expression and propidium iodide (PI) uptake (15). The parental XS106 DC line was cultured in 96 well plates with 0.5 ng/ml GM-CSF in the presence of each test drug and examined for 3 H-thymidine uptake on day 2. Regression curves were generated from the dose response datasets for YFP expression, growth inhibition, and cytotoxicity using the Regression Wizard (Sigmoidal dose response) function in the Sigma Plot program to calculate the “minimal effective

doses" (MEDs). The 54 drugs were clustered based on the MED values for the three functional parameters using the Gene Tree function in the GeneSpring program.

Effects of Type 1 drugs on DC phenotype and function

Bone marrow (BM)-derived DC cultures were propagated as before (18,19). After 24 h incubation with each test drug at 10^6 cells/ml, the cells were examined for surface phenotype within the CD11c⁺ populations and release of cytokines by ELISA. Following 24 h incubation with a test drug, BM-DCs were incubated for 10 min with 5 mg/ml of FITC-conjugated dextran (DX) (70 kD, Sigma) at 4°C or 37°C, washed extensively, and then examined for FITC signals by CD11c⁺ cells. After drug pretreatment, BM-DC preparations (derived from BALB/c mice) were washed 3 times and then co-cultured in 96 round-bottom well plates with splenic T cells purified from C57BL/6 mice (5×10^4 cells/well). The magnitude of T cell proliferation was assessed by ³H-thymidine uptake on day 4 (15). To test antigen cross-presentation, VBL-pretreated BM-DCs (derived from C57BL/6 mice) were pulsed with OVA protein (0.3 mg/ml) or OVA₂₅₇₋₂₆₄ peptide (1 µg/ml) for 1 h, washed extensively, and added to micro-cultures of CD8 T cells (5×10^4 cells/well) purified freshly from the OT-I transgenic mice. In some experiments, BM-DCs derived from the IAβ-EGFP knock-in mice (20) were co-cultured with VBL-pretreated BM-DCs from wild-type mice. Human DC cultures derived from the CD34⁺ cord blood progenitors were purchased from Mat Tek Corporation (21).

Statistical analysis

Each test drug was compared to vehicle alone control by a two-tailed student's *t*-test. All experiments were repeated at least three times to assess reproducibility.

Online supplemental material

Supplemental Figure S1 (VBL-induced phenotypic maturation of human DCs) and Supplemental Table S1 (Drugs screened in this study and their MED values).

RESULTS

Differential effects of anticancer drugs on DCs

From commonly used chemotherapeutic agents (17), we constructed a test library consisting of 12 topoisomerase inhibitors, 5 inhibitors of microtubule polymerization, 14 alkylating agents, 8 anti-metabolites, 4 platinum agents, 4 hormonal agents, and 7 others (Supplemental Table S1). Each of the 54 drugs was added at 5 different concentrations (0.1 to 10 µM) to micro-cultures of the DC biosensor clone in triplicates. After 16 h incubation, we examined YFP expression as an indicator of DC maturation (red lines in Fig. 1A). Several drugs induced significant YFP expression in dose-dependent manners. They included camptothecin sodium salt (CPT-Na), which was identified as a "hit" compound in our previous DC biosensor-based screening of a small compound library from the National Cancer Institute (15), as well as other topoisomerase inhibitors, such as doxorubicin (ADR), daunorubicin (DNR), epirubicin (EPR), idarubicin (IDR), mitoxantrone (MXT), and ellipticine (ELP). YFP expression was also triggered by anti-microtubule agents, including vinblastine (VBL), paclitaxel (PTX), and docetaxel (DCT). Two alkylating agents, mechlorethamine (HN2) and diaziquone (AZQ), and an anti-metabolite, cladribine (2-CdA), also induced YFP expression.

The same 54 drugs were also tested for their cytotoxicity by measuring PI uptake by the DC biosensor clone (green lines in Fig. 1A). Varying degrees of cytotoxicity were observed with all tested topoisomerase inhibitors, except for irinotecan (CPT-11) and etoposide (VP-16). Anti-microtubule inhibitors, VBL, PTX, vincristine (VCR), and vinorelbine (VRL), and a

retinoid, alitretinoin (9-cRA), also induced modest reduction in cell viability. Other drugs exhibited significant cytotoxicity in the tested concentration range.

As the third readout, we measured the impact on the parental XS106 DC growth primarily to validate biological activities of the tested drugs (blue lines in Fig. 1A). A total of 35 drugs, including all tested topoisomerase inhibitors and microtubule inhibitors, inhibited GM-CSF-dependent growth of XS106 DCs in dose-dependent fashions.

Classification of anticancer drugs based on their differential impacts on DCs

Our screening results unveiled a striking diversity among the tested anticancer drugs in their impacts on maturation, survival, and growth of the XS106 DCs. To interpret the data systematically, we calculated from each dose-dependence curve the “minimal effective dose” (MED) value for DC maturation, which was defined as the concentration required for producing 30% increase in YFP signals above the baseline level. Likewise, we calculated the MED values for DC killing and for DC growth arrest, defined as the concentrations causing 77% (i.e., 100/130) reduction in cell viability and ³H-thymidine uptake, respectively. Actual MED values of all tested drugs are described in Table 1.

We next employed the MED values as denominators to categorize the drugs based on their relative efficiencies to induce DC maturation, DC killing, and DC growth arrest (Fig. 1B). This approach enabled us to classify the 54 chemotherapeutic agents into: a) Type 1 drugs (15 indicated with red lines in Fig. 1B) that delivered DC maturation signals at concentrations causing only marginal DC death, b) Type 2 drugs (19 indicated with blue lines) that primarily inhibited DC growth, c) Type 3 drug (actinomycin D or AMD indicated with a purple line) that caused DC growth arrest and DC death without delivering DC maturation signals, and d) Type 0 drugs (19 indicated with black lines) that cause no substantial change at the tested concentrations. Anticancer drugs categorized as the Type 1 were of our particular interest because some of them might be used as “immunostimulatory” chemotherapeutics - they included nine topoisomerase inhibitors (CPT, CPT-Na, ADR, DNR, EPR, IDR, VP-16, MXT, and ELP), three anti-microtubule agents (VBL, PTX, and DCT), two alkylating agents (HN2 and AZQ), and a purine analog, 2-CdA.

Do Type 1 anticancer drugs indeed induce DC maturation?

Although the DC biosensor system provides a time- and cost-efficient assay platform, it has two major limitations. First, the indicator we employed (i.e., IL-1 β promoter activation) may not fully reflect the state of DC maturation. Secondly, the DC preparation we examined (i.e., XS106 DC line) may not fully represent *bona fide* DCs. In fact, the magnitude of YFP expression inducible by a given stimulus did not correlate with its actual impact on BM-DC preparations (15). We next sought to determine whether some of the Type 1 anticancer drugs would induce characteristic changes known to accompany DC maturation. In this regard, we observed previously that CPT-Na, which was identified as a hit from the NCI small compound library, induces phenotypic maturation of murine BM-DCs without causing robust cytokine production or augmenting their T cell-stimulatory capacity (15). Thus, we tested the remaining 14 Type 1 drugs in this study for their effects on BM-DCs. Each drug was tested at a predetermined concentration, i.e., the highest MED value for DC maturation selected from three independent screening experiments. DNR and HN2, which were found to kill large numbers of BM-DCs at the above defined concentrations, were tested at arbitrarily chosen concentrations of 0.3 and 0.6 μ M, respectively.

BM-DCs were cultured for 24 h with each test drug and then examined for surface expression of CD40, CD80, CD86, and MHC II. A microtubule inhibitor, VBL, appeared to elevate the expression of all four markers of DC maturation, without affecting cell viability measured by

PI uptake (Fig. 2A). We performed the experiment with triplicate samples and scored the observed difference as “upregulation” only when a given drug induced a statistically significant ($P < 0.05$) and biologically substantial ($>30\%$) increase in the median fluorescence intensity value above the baseline level. Three drugs, VBL, PTX, and VP-16, fulfilled the dual criteria for upregulating all four the phenotypic markers for DC maturation (indicated in red in Fig. 3A). Other drugs that were found to upregulate CD40 expression (CPT, ADR, IDR, ELP, HN2, and AZQ) failed to elevate the expression of CD80, CD86, or MHC II. The viability of BM-DCs remained mostly unchanged after 24 h incubation with any drug at the tested concentration.

Supernatants collected from the above BM-DC cultures were then examined for IL-1 β , IL-6, IL-12 p40, and TNF α (Fig. 3B). Once again, we employed the same dual criteria to define a statistically significant ($P < 0.05$) and biologically substantial ($>30\%$) change in cytokine production. Nine drugs induced upregulated production of IL-1 β protein by BM-DCs, and two downregulated IL-1 β production. Likewise, production of other cytokines was upregulated by some of the Type 1 drugs. Interestingly, MXT, which failed to induce phenotypic maturation, upregulated the production of all tested cytokines. VBL, which induced full phenotypic maturation, upregulated the production of IL-1 β , IL-6 and IL-12, while downregulating TNF α production. These findings further illustrated differential impacts of Type 1 drugs on different functional parameters of DC maturation.

The most crucial functional property of mature DCs is the ability to activate immunologically naïve T cells efficiently. Thus, we pretreated BM-DCs with each Type 1 drug and then tested their efficiency to activate allogeneic T cells. DCs pretreated with CPT, ELP, VBL, PTX, DCT, HN2, AZQ, or 2-CdA exhibited significantly ($P < 0.01$) augmented T cell-stimulatory capacity (Fig. 2B). By contrast, DCs pretreated with DNR showed a significantly reduced ability to activate allogeneic T cells. The remaining Type 1 drugs (ADR, EPR, IDR, VP-16, and MXT) induced no detectable changes in the T cell-stimulatory potential.

In summary, not all Type 1 anticancer drugs uniformly induced a full spectrum of maturational changes in DCs (Fig. 4). Instead, we observed marked heterogeneity in their abilities to elevate surface expression of CD40, CD80, CD86, and MHC II, to trigger production of IL-1 β , IL-6, IL-12, and TNF α , and to augment T cell-stimulatory capacity of DCs. It should be noted here that each drug produced at least one of these characteristic changes. Most notably, VBL was found to produce all the maturational changes of DCs (except for TNF α production) in BM-DCs.

Pharmacological effects of VBL on DCs

Having identified VBL as a prototypic Type 1 anticancer drug, we next characterized its effects on DCs further. In dose-dependency experiments, VBL ranging from 0.1 to 1.0 μM induced IL-1 β , IL-6, and IL-12 p40 production and elevated CD40, CD80, CD86, and MHC II expression (Fig. 5A). To begin to understand mechanisms by which VBL induces DC maturation, we determined whether VBL-pretreated BM-DCs (from wild-type mice) may indirectly activate second BM-DC preparations (derived from the IA β -EGFP knock-in mice). The EGFP/CD11c $^{+}$ DC populations showed CD86 upregulation after co-culturing with VBL-pretreated DCs (Fig. 5B), and the observed CD86 expression was upregulated further by adding VBL directly to the co-cultures of VBL-pretreated DCs and non-treated DCs (Fig. 5C). These results suggest dual mechanisms of VBL action, i.e., direct and indirect DC activation. With regard to mechanisms for the indirect pathway, the cytokines being released from VBL-pretreated DCs (Fig. 3B) may play functional roles.

Although it is generally believed that DCs efficiently incorporate exogenous antigens only in the immature state (22), West *et al.* demonstrated that BM-DCs exhibit augmented uptake of

FITC-DX following stimulation with various toll-like receptor (TLR) ligands (23). Thus, we measured FITC-DX uptake as an additional functional parameter. Control BM-DCs treated with vehicle alone showed modest FITC-DX uptake at 37°C and minimal surface binding at 4°C. By marked contrast, DCs treated with VBL showed an augmented endocytic capacity (Fig. 6A). Quantitative analysis revealed 10-fold improvement of FITC-DX uptake after VBL treatment (Fig. 6B). Importantly, none of the other tested Type 1 drugs exhibited such striking effects, except for PTX augmenting FITC-DX incorporation modestly. These observations implied that VBL might promote cross-presentation of exogenous antigens to CD8 T cells. In fact, VBL-treated BM-DCs were far more efficient than vehicle-treated DCs in presenting OVA protein to CD8 T cells purified from the OT-I T cell receptor transgenic mice, in which a majority of CD8 T cells recognize the OVA₂₅₇₋₂₆₄ peptide presented on H2-K^b (Fig. 6C). VBL treatment also augmented, albeit modestly, their ability to present OVA₂₅₇₋₂₆₄ peptide to the same OT-I CD8 T cells.

Although beyond the scope of the present study, we tested the effects of VBL on human DCs using commercially available DC preparations derived from CD34⁺ progenitors in the cord blood. Upon exposure to VBL at 0.3 μM, human DCs (as defined by CD1a expression in those cultures) elevated surface expression of MHC II, CD40, CD80, and CD86, elaborated IL-6, IL-8, RANTES, and MIP-1α, and exhibited robust uptake of FITC-DX by human DCs after VBL treatment (Supplemental Fig. S1). These *in vitro* observations demonstrated the potential of VBL to trigger phenotypic and functional maturation of human DCs as well.

DISCUSSION

Our unbiased functional screen of 54 chemotherapeutic agents has unveiled striking diversity in their pharmacological impact on maturation, survival, and growth of DCs. Based on the concentrations required to affect the three functional parameters, we categorized the drugs into four classes. To our knowledge, this is the first published study in which diverse anticancer drugs are compared in parallel for their effects on DCs. Considering the important roles played by DCs in initiating and regulating host immune responses to cancer (1,7), our screening results provide essential information that will allow rational selection of chemotherapeutic agents for ultimate clinical outcome.

Suppression of host immunity is one of the major adverse effects of chemotherapy. Contrary to this general notion, 15 anti-cancer drugs were categorized as Type 1 in our DC biosensor screening. These drugs were subsequently confirmed to induce at least one of the characteristic maturational changes in BM-DCs. Interestingly, most of the tested topoisomerase inhibitors (9/12) and anti-microtubule agents (3/5) were categorized as Type 1, while the remaining drugs in these two families were categorized as Type 2. By contrast, only a few tested alkylating agents (2/14), an anti-metabolite, and none of platinum agents or hormonal agents satisfied the criteria for Type 1. These observations may suggest intriguing correlation between the functional property of delivering DC maturation signals and the pharmacological mechanisms of action among the tested chemotherapeutic agents.

VBL was regarded as the most prominent inducer of DC maturation among the tested Type 1 drugs; it elevated CD40, CD80, CD86, and MHC II expression, triggered IL-1β, IL-6, and IL-12 p40 production, and augmented the capacity to activate allogeneic T cells. Interestingly, VBL appears to induce DC maturation both directly and indirectly. VBL markedly improved the abilities of BM-DCs to incorporate FITC-DX and to cross-present OVA protein to CD8 T cells. Our findings may first appear contradictory to the general notion of VBL as a chemotherapeutic drug with immunosuppressive potentials. Indeed, VBL has been reported to inhibit mitogen-induced lymphocyte activation (24), induction of cytotoxic T cell activity (25), tumoricidal potential of macrophages (26), natural killer cell-mediated cytotoxicity (27),

antibody-dependent cell-mediated cytotoxicity (28), humoral immune responses (29), cellular immune protection against microbial infection (30), and host anti-tumor immunity (31). However, most of those immunosuppressive properties were observed for VBL at relatively high concentrations and/or after repeated administrations. Our data demonstrated that VBL at relatively low concentrations (0.1-1 μ M) induced phenotypic and functional maturation of mouse and human DCs. We observed recently that local injection of a small amount of VBL is sufficient to trigger *in situ* maturation of skin resident DCs and boost humoral and cellular immune responses to a model antigen in mice. When injected directly into B16 melanoma in a small amount, VBL elicited a marked CTL activity against melanoma targets and interrupted otherwise progressive growth of B16 melanoma (Tanaka et al., accompanying paper). Thus, we suggest that VBL produces opposing immunological outcomes depending upon administration doses. To the best of our knowledge, this is the first report documenting the ability of VBL to trigger maturation of DCs.

Our findings with VBL are consistent with our previous results - two microtubule inhibitors, colchicine and podophyllotoxin, were identified as “hits” by DC biosensor-based screening of 880 FDA-approved drugs (15). With regard to underlying mechanisms, colchicine and podophyllotoxin both activated the NF κ B pathway in DCs. As observed with VBL, they both induced production of IL-1 β , IL-6, and IL-12, but not TNF α . Interestingly, a synthetic microtubule inhibitor, CC-5079, was reported to suppress TNF α production by inhibiting phosphodiesterase type 4, an essential cyclic AMP-metabolizing enzyme known to be involved in lipopolysaccharide (LPS)-activated TNF α responses (32). VBL is one of the classic *Vinca* alkaloids that have greatly contributed to the clinical success of chemotherapy over the last four decades (33). At high concentrations, these agents directly interfere with proper spindle microtubule formation, thereby blocking cell mitosis and eventually leading to apoptosis. The *Vinca* alkaloids also stabilize microtubule dynamics at low concentrations, thereby affecting various cellular activities. Thus, we suggest that partial and temporal disruption of intracellular microtubule networks may be sensed by DCs as intrinsic danger signals.

In summary, we have screened a variety of chemotherapeutic agents in the DC biosensor system, categorized them based on their differential impacts on three functional parameters of DCs, and identified several structurally unrelated drugs capable of triggering DC maturation. Since DCs play crucial roles in regulating host immunity against tumor, our screening results will provide essential datasets for rational selection and combination of anticancer drugs for treatment of cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

- (1). Dhodapkar MV, Dhodapkar KM, Palucka AK. Interactions of tumor cells with dendritic cells: balancing immunity and tolerance. *Cell Death Differ* 2008;15:39–50. [PubMed: 17948027]
- (2). Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004;22:329–60. [PubMed: 15032581]
- (3). Kim R, Emi M, Tanabe K, Arihiro K. Tumor-Driven Evolution of Immunosuppressive Networks during Malignant Progression. *Cancer Res* 2006;66:5527–36. [PubMed: 16740684]
- (4). Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005;5:263–74. [PubMed: 15776005]

- (5). O'Neill DW, Adams S, Bhardwaj N. Manipulating dendritic cell biology for the active immunotherapy of cancer. *Blood* 2004;104:2235–46. [PubMed: 15231572]
- (6). Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol* 2005;5:296–306. [PubMed: 15803149]
- (7). Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007;449:419–26. [PubMed: 17898760]
- (8). Lake RA, Robinson BW. Immunotherapy and chemotherapy--a practical partnership. *Nat Rev Cancer* 2005;5:397–405. [PubMed: 15864281]
- (9). Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol* 2008;8:59–73. [PubMed: 18097448]
- (10). Casares N, Pequignot MO, Tesniere A, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005;202:1691–701. [PubMed: 16365148]
- (11). 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;109:4839–45. [PubMed: 17299090]
- (12). Proietti E, Greco G, Garrone B, et al. Importance of cyclophosphamide-induced bystander effect on T cells for a successful tumor eradication in response to adoptive immunotherapy in mice. *J Clin Invest* 1998;101:429–41. [PubMed: 9435316]
- (13). Ghiringhelli F, Larmonier N, Schmitt E, et al. CD4⁺CD25⁺ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol* 2004;34:336–44. [PubMed: 14768038]
- (14). Mullins DW, Burger CJ, Elgert KD. Paclitaxel enhances macrophage IL-12 production in tumor-bearing hosts through nitric oxide. *J Immunol* 1999;162:6811–8. [PubMed: 10352302]
- (15). Mizumoto N, Gao J, Matsushima H, Ogawa Y, Tanaka H, Takashima A. Discovery of novel immunostimulants by dendritic cell-based functional screening. *Blood* 2005;106:3082–9. [PubMed: 16002424]
- (16). Matsue H, Matsue K, Walters M, Okumura K, Yagita H, Takashima A. Induction of antigen-specific immunosuppression by CD95L cDNA-transfected “killer” dendritic cells. *Nat Med* 1999;5:930–7. [PubMed: 10426318]
- (17). Chu, E.; Takimoto, CH.; McLeod, HL., et al. Pharmacology of Cancer Chemotherapy. In: DeVita, VT., Jr.; Hellman, S.; Rosenberg, SA., editors. *Cancer: Principles and Practice of Oncology*. Vol. 7th Edition. Lippincott Williams & Wilkins; Philadelphia: 2005. p. 307–422.
- (18). Matsue H, Yang C, Matsue K, Edelbaum D, Mummert ME, Takashima A. Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and dexamethasone) on bidirectional dendritic cell-T cell interaction during antigen presentation. *J Immunol* 2002;169:3555–64. [PubMed: 12244145]
- (19). Mizumoto N, Kumamoto T, Robson SC, et al. CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness. *Nat Med* 2002;8:358–65. [PubMed: 11927941]
- (20). Boes M, Cerny J, Massol R, et al. T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* 2002;418:983–8. [PubMed: 12198548]
- (21). Mizumoto N, Tanaka H, Matsushima H, Vishwanath M, Takashima A. Colchicine promotes antigen cross-presentation by murine dendritic cells. *J Invest Dermatol* 2007;127:1543–6. [PubMed: 17301837]
- (22). Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. *J Exp Med* 1995;182:389–400. [PubMed: 7629501]
- (23). Bot FJ, Schipper P, Broeders L, Delwel R, Kaushansky K, Lowenberg B. Interleukin-1 α also induces granulocyte-macrophage colony-stimulating factor in immature normal bone marrow cells. *Blood* 1990;76:307–11. [PubMed: 1695108]
- (24). Greene WC, Parker CM, Parker CW. Colchicine-sensitive structures and lymphocyte activation. *J Immunol* 1976;117:1015–22. [PubMed: 956646]

- (25). Dye ES, North RJ. Adoptive immunization against an established tumor with cytolytic versus memory T cells. Immediate versus delayed onset of regression. Transplantation 1984;37:600–5. [PubMed: 6427998]
- (26). Sharma SD, Piessens WF. Tumor cell killing by macrophages activated in vitro with lymphocyte mediators. III. Inhibition by cytochalasins, colchicine, and vinblastine. Cell Immunol 1978;38:276–85. [PubMed: 209902]
- (27). Katz P, Zaytoun AM, Lee JH Jr. Mechanisms of human cell-mediated cytotoxicity. III. Dependence of natural killing on microtubule and microfilament integrity. J Immunol 1982;129:2816–25. [PubMed: 6890568]
- (28). Strom TB, Garovoy MR, Carpenter DB, Merrill JP. Microtubule function in immune and nonimmune lymphocyte-mediated cytotoxicity. Science 1973;181:171–3. [PubMed: 4541240]
- (29). Moav N, Steinberg L, Frensdorff A. Effect of vinblastine on the early events in the humoral immune response of mice to SRBC. Int Arch Allergy Appl Immunol 1976;52:257–65. [PubMed: 797670]
- (30). McGregor DD, Logie PS. The mediator of cellular immunity. VI. Effect of the antimetabolic drug vinblastine on the mediator of cellular resistance to infection. J Exp Med 1973;137:660–74. [PubMed: 4144033]
- (31). Klenner P, Bubenik J, Jandjsek J. Influence of Vinblastine on antitumor immunity in mice. Neoplasma 1973;20:239–42. [PubMed: 4725513]
- (32). Zhang LH, Wu L, Raymon HK, et al. The synthetic compound CC-5079 is a potent inhibitor of tubulin polymerization and tumor necrosis factor- α production with antitumor activity. Cancer Res 2006;66:951–9. [PubMed: 16424030]
- (33). Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer 2004;4:253–65. [PubMed: 15057285]

Figure 1. Classification of anticancer drugs based on their differential impacts on DCs
(A) Each drug was tested at 5 different doses (0.1-10 μM) for its efficacies to elevate YFP expression by the DC biosensor clone (red lines), to kill the same DC clone (green), and to inhibit GM-CSF-dependent growth of the parental XS106 DC line (blue). Data shown are a representative set of results (means $\pm$ SD, $n = 3$) from three independent experiments. Note that YFP expression levels are shown in different scales for some drugs. **(B)** From the above dose-dependency curves, the MED values were determined for DC maturation (YFP expression), growth arrest (^3H -thymidine uptake), and killing (PI uptake). These values, converted into different colors as indicated in the color scale, were then employed as quantitative denominators for clustering the drugs. Mechanisms of action for individual drugs are indicated in different colors as shown in Table 1.

Figure 2. Effects of VBL and other Type 1 anticancer drugs on BM-DCs

(A) BM-DCs were incubated for 24 h with 0.3 μ M VBL (filled histograms) or vehicle alone (open) and then examined for surface expression of the indicated markers and for PI uptake within the CD11c⁺ populations. (B) BM-DCs (from BALB/c mice) were incubated for 24 h with the indicated drugs (closed circles) or vehicle alone (open circles). After extensive washing, the DC preparations were cultured at the indicated numbers with T cells purified from C57BL/6 mice. Data shown are the means $\pm$ SD ($n = 3$) of ³H-thymidine uptake. Statistically significant differences compared with the vehicle-treated control are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$).

Figure 3. Relative efficacy of Type 1 anticancer drugs to promote phenotypic maturation and cytokine production by BM-DCs

(A) BM-DCs were incubated for 24 h with each drug or vehicle alone in triplicates and then examined for surface expression of the indicated markers and for PI uptake within the CD11c⁺ populations. (B) Supernatants harvested from the same cultures were examined for the indicated cytokines. Broken lines indicate the baseline values observed after treatment with vehicle alone. Statistically significant ($P < 0.05$) and biologically relevant (>30%) increase above or decrease below the baseline value is indicated in red or blue, respectively.

	CD40	CD80	CD86	MHC II	IL-1 β	IL-6	IL-12	TNF α	MLR
CPT									
ADR									
DNR									
EPR									
IDR									
VP-16									
MXT									
ELP									
VBL									
PTX									
DCT									
HN2									
AZQ									
2-CdA									

Figure 4. Summary of differential impact of Type 1 anticancer drugs on BM-DCs

Impacts of the tested Type 1 anticancer drugs on different phenotypic and functional parameters of DC maturation are summarized in the checkerboard, in which statistically significant and biologically relevant increases or decreases are indicated in red or blue, respectively.

Figure 5. Dose-dependency and mechanisms of VBL-induced DC maturation

(A) BM-DCs were incubated for 24 h with VBL at the indicated concentrations and then examined for production for the indicated cytokines and expression of the indicated markers. Data shown are the means $\pm$ SD ($n = 3$) of cytokine concentrations and median fluorescence intensities. Statistically significant differences compared with the vehicle-treated control are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$). (B) BM-DCs derived from wild-type mice were pre-incubated for 24 h with 1 μ M VBL or vehicle alone, washed them extensively, and added to cultures of BM-DCs derived from the IA β -EGFP knock-in mice. After additional 24 h incubation in the presence or absence of freshly added VBL (1 μ M), the EGFP/CD11c⁺ DC populations were analyzed after staining with anti-CD86 mAb (filled histograms) or an isotype-matched control IgG (open). (C) CD86 expression levels in the above experiment are compared among four experimental conditions ($n = 3$, * $P < 0.05$, ** $P < 0.01$).

Figure 6. VBL-induced augmentation of FITC-DX uptake and antigen cross-presentation by BM-DCs

(A) BM-DCs were incubated for 24 h with 0.3 μM VBL (filled histograms) or vehicle alone (open), incubated with FITC-DX at 4°C or 37°C, and then examined for FITC fluorescent signals within the CD11c⁺ populations. (B) Following 24 h incubation with a test drug, BM-DCs were examined for FITC-DX uptake (mean fluorescence intensity) within the CD11c⁺ populations. Data shown are the means ± SD (n = 3) of relative activities (% of control) compared to the vehicle-treated control. Statistically significant ($P < 0.05$) and biologically relevant (>30%) increase above or decrease is indicated in red or blue, respectively. (C) BM-DCs were incubated for 24 h with 0.3 μM VBL (circles) or vehicle alone (triangles). The DC preparations were pulsed for 1 h with intact OVA protein (0.3 mg/ml), OVA₂₅₇₋₂₆₄ peptide (10 μg/ml), or PBS alone, washed extensively, and added at the indicated cell numbers to microcultures of CD8 T cells (5×10^4 cells/well) purified from the OT-I transgenic mice. Data shown are the means ± SD (n = 3) of ³H-thymidine uptake. Statistically significant differences compared with the vehicle-treated control are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$).