Doxorubicin, a WHO listed essential medicine, is used primarily as a combination of taxane/anthracycline (doxorubicin)/cyclophosphamide (TAC), for treating malignant diseases including triple-negative breast cancer (TNBC). TNBC is a subtype of breast cancer categorized by deficient estrogen receptor, progesterone receptor, and epidermal growth factor receptor HER-2 [1]. TNBC is challenging to treat due to its genotype and phenotype heterogeneity [2-5], aggressiveness, recurrence [6-10], and resistance to existing therapies. Roughly, 25-45% TNBC patients receiving TAC as preoperative therapy achieve complete response and excellent long-term prognosis [11], while patients who fail TAC have poor prognosis and few therapeutic choices [2]. Specifically, doxorubicin-responsive TNBC patients achieve disease-free status and superb long-term prognosis while non-responders face chemoresistance, metastasis, and limited post-doxorubicin options. To increase curable efficacy and counter drug resistance in TNBC, the discovery of clinically relevant, assayable targets and novel mechanism-based therapies are imperative. Unmet clinical needs that constitute the focus of this communication include: How can doxorubicin–response be improved and therapeutic targets be unraveled? What controls and counters the chemoresistance in treated subjects?

**PD-L1 expression and immune escape**

The immune checkpoint transmembrane protein, programmed cell death–ligand 1 (PD-L1) is expressed in a myriad of immune and tumor cells [12,13]. Tumor PD-L1 induces the demise of infiltrating cytotoxic T cells in the tumor environment by binding to receptor PD-1 on T cells [13,14]. As such, PD–L1 acts as a protective shield to counter the host immune surveillance mechanism. In certain cancer cell types, high expression of tumor cell PD-L1 correlates with clinical efficacy in the use of checkpoint blockade therapy where monoclonal antibodies are deployed to disrupt the PD-1/PD-L1 axis [15-20]. Disease- and progression–free survival to immune checkpoint blockade therapy has been shown in melanoma, lung, renal and breast carcinomas, in a small percentage of patients expressing high but not low/undetectable PD-L1 tumors [21-24].

Resistance to doxorubicin and metastasis in tumor cells is linked to the activation of PD–L1 [25]. PD–L1 in tumor cells regulates immunosuppression and tumor escape. An altered tumor immunity, drug resistance and increase in metastasis are among ways by which cancer cells elude chemotherapy [26,27]. We hypothesize that PD–L1 is a novel, cancer stage-independent marker to identify candidate cancer patients including TNBC who present clinical chemoresistance. Testing this hypothesis could help unravel existing gaps in treatment of TNBC, viz. tumor heterogeneity, lack of response to host immunity, and resistance to existing therapies. The outcome is significant because it probes the regulation of PD–L1 in doxorubicin–resistant tumor cells using TNBC as example, advances our knowledge of drug induced chemo- and immune resistance via upregulation of tumor PD–L1, and facilitates the development of anti-PD–L1 approaches to prevent or treat cancer. We surmise that TNBC patients displaying doxorubicin resistance and metastasis may be amenable to treatment...
using the immune checkpoint blockade therapy to disrupt the interaction between PD1/PD-L1, thus offering a target-, stage- and cancer type-directed therapy for patients who fails chemotherapy. The efficacy to immune checkpoint inhibitors as gauged by disease-, progression-free survival has been shown in melanoma, lung, renal and breast carcinomas [21-24].

**Novel regulation of PD-L1 by NF-κB, subject to modulation by NQO2**

The mechanism by which up regulation of PD-L1 alters response to host immunity in tumors, evident by resistance to treatment in patients has not been elucidate. Similarly, how PD-L1 in TNBC is regulated by doxorubicin remains unknown. In our published study, we show that at ≥IC50 resveratrol, a NRH:quinone oxidoreductase (NQO2) inhibitor increases PD-L1 in colon and breast cancer cells including TNBC by activation of nuclear factor-κappa B (NF-κB) (28). In EMT6 syngeneic breast tumor mouse model studies, we found that doxorubicin also induces PD-L1 [29]. We recently showed that in cancer cells treated with supra-pharmacological NQO2 inhibitor doxorubicin, a viable residual cell population remains and expresses greatly elevated PD-L1 accompanied by the activation of NF-κB and induction of DDR (DNA damage response) [29]. These observations suggest that in tumor cells, the acquisition of doxorubicin-resistance occurs in parallel with DDR- and NF-κB-mediated induction of PD-L1. As such, to improve the efficacy of doxorubicin, co-suppression of drug resistance and PD-L1 expression using anti-PD-L1 therapy seems a desirable, yet-to-be explored therapeutic option. These observations are significant: NF-κB positively controls PD-L1 while NQO2 is inversely associated with an increase in the risk of breast cancer. We hypothesize drug/treatment induced tumor PD-L1 may be associated with drug resistance. In addition, inhibitor of NQO2 can facilitates PD-L1-mediated tumor escape of host immunity and modulate doxorubicin-resistance via NF-κB, provides gain-of-growth by treatment induced resistant tumor cells. It strongly suggests the use of anti-PD-L1-antibody – an underpinning of immune checkpoint inhibitors, as a therapeutic option for TNBC patients who fail the doxorubicin therapy. Tumor PD-L1 may serve as a drug-resistant biomarker and theranostic indicator for cancer patients e.g. TNBC. In addition, PD-L1 promotes tumor cell survival, and escape from host immunity, thus, immune checkpoint blockade therapy can appropriately treat chemoresistance mediated by drug e.g. doxorubicin induced tumor PD-L1 using combination versus single-agent therapy is illustrated (Figure 1). The underlying tenets are: (i) patients expressing low/no PD-L1 tumor cells are candidates for immune checkpoint-responsiveness, via the upregulation of PD-L1, (ii) NF-κB signaling mediates the induction of PD-L1 as well as tumor survival, and (iii) the non-conventional phase II enzyme NQO2 characterized [30,31], plays the novel role of modulating NF-κB response/signaling to facilitate/enhance the elimination of tumor cells by host antitumor immunoregulatory processes.

**References**


**Figure 1:** Schemes illustrate (1) drug induced upregulation of PD-L1 resulted in drug resistance and tumor survival and (2) NQO2 mediates control of tumor PD-L1, tumor cell survival/escape via NF-κB signaling. Tumor acquired resistance can become immune blockade-responsive by upregulating tumor PD-L1, with NF-κB as initiator, and NQO2 as the NF-κB mediator.

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