A FOCUS ON PD-L1 IN HUMAN MELANOMA

Running title: Combining inhibitors of PD-L1 and immunotherapy

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There are no potential conflicts of interest

Treatment of metastatic melanoma with inhibitors of the BRAF V600 oncogene in melanoma has been limited by the development of resistance. Combining the BRAF inhibitors with immunotherapy may prolong the response but will acquisition of resistance to BRAF inhibitors also make melanoma cells resistant to immunotherapy?

In this issue of Clinical Cancer Research, Jiang et al (1) and colleagues report that melanoma cells that have developed resistance to the selective BRAF inhibitor Vemurafenib had increased expression of the programmed death receptor ligand 1 (PD-L1). PD1 is a receptor that is acquired by T cells after chronic stimulation and acts as an important checkpoint inhibitor to downregulate the function of T cells in assays of cytokine and cytotoxic activity.(2) PD1 expression increases with progression of melanoma and may contribute to the escape of melanoma from host immune responses.(3) The PD-L1 ligand for PD1 is expressed on some melanoma and also on stromal cells around melanoma and increases with progression of the melanoma.(4, 5). Phase 1 studies on 94 patients with metastatic melanoma treated with a monoclonal antibody (MAb) against PD1 (BMS936558) showed relatively high response rates that appeared durable.(6) Importantly, responses appeared confined to patients who had cancers that expressed PD-L1 in that responses were seen in 9 of 25 patients with PD-1 expressing cancers but there were no responses in 17 patients with PD-L1 negative tumors.

These results raised the possibility that PD-L1 expression on cancers may be a biomarker of response to treatment with MAb to PD1 and have focused attention on factors regulating the expression of PD-L1 on tumors. One study raised the possibility that PD-L1 may be induced by immune responses against the melanoma and represent an adaptive immune resistance mechanism that allows the tumor to escape immune destruction. This interpretation was supported by a close correlation between PD-L1 expression and the presence of tumor infiltrating lymphocytes (TILs) as well as induction of PD-L1 on
melanoma in-vitro by IFN gamma(5). Involvement of the RAS/RAF/MEK pathway in melanoma was shown by studies showing that melanocytes transfected with V600E or V600E mutated melanoma cells produced Vemurafenib inhibitable production of IL1. Moreover fibroblasts grown from tumor biopsies were able to inhibit cytotoxic T lymphocyte (CTL) activity against melanoma when they were treated beforehand with IL1. The inhibitory activity of the fibroblasts was attributed to the induction of PD-L1 and PD-L2 on the fibroblasts as well as upregulation of COX 2.(7)

The studies of Jiang et al add another dimension to these studies in showing that PD-L1 is more strongly upregulated in melanoma cells with acquired resistance to Vemurafenib (and most probably other selective BRAF inhibitors) than was evident in the parental V600 mutated melanoma. Given that the MEK/ERK pathway was also activated in the parental cells, the results implied that additional pathways perhaps related to the resistance to BRAF inhibitors were upregulating PD-L1 expression. The authors provide evidence that activation of the stress related MAPK, JNK is at least partially responsible for the increased expression of PD-L1 via upregulation of the transcription factor c-Jun. The transcription factor STAT3 also appeared to contribute to upregulation of PD-L1 but it was not clear which signal pathway may be involved in activating STAT3. Knockdown of either c-Jun or STAT3 was observed not to induce apoptosis in the melanoma cells whereas inhibition of either MEK of JNK resulted in substantial induction of apoptosis. Paradoxical hyperactivation of the MEK /ERK pathway is a feature of many cells that are resistant to selective BRAF inhibitors particularly when there are activating mutations of NRAS(8, 9). The importance of this hyperactivation of MEK was born out in these studies by the induction of apoptosis as well as reduction in PD-L1 expression by inhibition of MEK.

These findings are particularly relevant firstly, to the current discussions concerning the optimum sequencing of combining immunotherapy with selective BRAF inhibitors in treatment of patients with mutated BRAF melanoma. There is largely anecdotal evidence(10) that patients failing treatment with Vemurafenib do not respond to immunotherapy with Ipilimumab and this has favoured arguments for treatment with Ipilimumab prior to treatment with selective BRAF inhibitors. This question is being addressed in the ECOG 1612 trial on the best sequencing of these agents but the strong upregulation of PD-L1 in the Vemurafenib resistant cells shown by Jiang et al would explain why Ipilimumab might be ineffective against such cells. Secondly it is also possible that the downregulation of PD-L1 by cotreatment with inhibitors of both BRAF and MEK shown in the Jiang et al study may have contributed to the better progression free survival (PFS) of patients treated in the randomized phase II study comparing Dabrafenib alone( PFS 5.8 mths) with the combination of Dabrafenib and the MEK inhibitor Trametinib (PFS 9.4mths)(11).ie lower PD-L1 levels in the latter may have contributed to more effective immune responses in the latter.

Thirdly the results would appear to have implications for treatment of melanoma with MAbs against PD1 as illustrated in figure1. As discussed elsewhere by Pardoll and others PD-L1 expression on cancer cells may be an adaptive (resistance) response to immune attack and release of IFN gamma by tumor infiltrating lymphocytes(TILS) or be due to constitutive expression as a result of oncogenic processes such as those resulting from V600 BRAF mutations(12). Patients with melanoma that have adaptive PD-L1 expression that is blocked by anti PD1 MAbs may benefit from additional stimulation of immune
responses eg with vaccines or treatment with MAbs against CTLA. The absence of TILs in the second group of patients having BRAF V600 melanoma and constitutive PD-L1 expression is considered most likely due to release of immune inhibitory factors from melanoma cells. Such patients may therefore benefit from inhibitors of the signal pathways identified in the Jiang et al study with an emphasis on those that induce apoptosis. This appeared to be the MEK and JNK pathways but further study is needed to better define the apoptotic mechanisms involved. Block of the release of inhibitory factors would then be combined with immunotherapy with agents such as vaccines or MAbs against CTLA4. Patients with melanoma having wildtype BRAF and no TILs would constitute a third group where the absence of TILs would still be due to release of immune inhibitory factors by melanoma. The pathways involved in the latter are the subject of much interest and include activation of the transcription factor NF-κB. In broad principal however the treatment options would be to use anti PD1 to remove the inhibitory effect of PD-L1 and combine this with agents that block release of factors that inhibit immune responses together with agents that induce cytotoxic T lymphocyte (CTL) responses against the melanoma. The challenge will be to find inhibitors that block release of immune inhibitory factors from melanoma that do not also inhibit the activity of CTL.

Figure 1

Immune responses by CTL against melanoma depend on interaction of T cell receptors with melanoma antigens on MHC antigens on melanoma together with co stimulation by ligand receptor interactions like CD28 and CD80.

In the adaptive model IFN gamma from CTL upregulates a number factors in melanoma cells which provide feedback resistance to immune attack. This includes indolamine 2,3 dioxygenase that depletes tryptophan but most importantly PD-L1 which interacts with PD1 on CTL to downregulate their activity. Blocking PD1 may be sufficient to induce partial responses but responses may be further enhanced by additional immunotherapy.

In the constitutive PD-L1 model there is a paucity of TILs and PD-L1 is upregulated by the oncogenic process which may also be responsible for inhibiting infiltration and activation of TILs against the melanoma. Blocking PD1 by itself maybe ineffective unless the oncogenic process is also inhibited. Inhibitors of BRAF and MEK maybe sufficient for this but the effects of this combination on CTL needs further analysis. In melanoma cells wildtype for BRAF further study is needed to identify agents that may block release of inhibitory factors against immune responses but not the activity of CTL. In both models additional forms of immunotherapy could be expected to improve responses.
References


Figure 1:

1 Adaptive model

Melanoma cells

- PD-L1
- Anti PD1
- CD28
- IFN δ
- MHC/antigen

Cotreatment

- Melanoma vaccines or anti-CTLA4

2 Constitutive model

Due to paradoxical activation

BRAF V600

- PD-L1
- Product of immune inhibitory factors

Cotreatment

- Anti PD1 plus Melanoma vaccines or anti CTLa4 to activate T cells

3 Constitutive model wildtype for BRAF

- Immune inhibitory factors
- Signal pathway inhibitors/epigenetic modifiers

Production of immune inhibitory factors blocked

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