Are PD-1 and PD-L1 relevant targets in paediatric malignancies?

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In the last 5 years, immune checkpoint inhibitors have shown remarkable success in adult malignancies. In particular, monoclonal antibodies that block the interaction between programmed death ligand 1 (PD-L1), present on the surface of tumor or antigen-presenting cells, and programmed death 1 (PD-1), present on the surface of chronically activated lymphocytes, have shown impressive tumour responses in a wide range of cancers, including those that are not traditionally considered to be immunogenic. Such agents potentially offer an "off the shelf" immunotherapy, unleashing endogenous anti-tumour immune responses to generate durable tumour control in a significant number of patients. A number of these agents have now been licensed as first or second line therapeutics. Some of the most impressive results to date have been seen when antibodies targeting the PD-1 are combined with those targeting the immune checkpoint molecule CTLA-4, with objective response rates in 57% in patients with metastatic melanoma [1]. Across all tumour types, the most consistent response biomarker appears to be the expression of PD-L1 on tumour cells. However, although higher responses are reported in patient with tumours with immunohistochemically detectable PD-L1, patients whose tumours do not express PD-L1 can still have impressive responses to PD-1 blockade. Recent reports have suggested that response is also likely to be predicted by the 'mutational burden' of the tumour, which is consistent with the significant response rates seen in highly mutated tumours such as melanoma and non-small cell lung cancer [2].

Although there are now in excess of 100 trials in progress of this class of agents in adults, the first paediatric studies have only opened recently, and there is as yet no paediatric clinical data reported. The relatively low mutational burden in most paediatric cancers has generated some caution as to whether they will prove as effective as in adult cancers. Nevertheless, although most paediatric cancers lack true neoantigens, numerous tumour antigens have been identified, and in some instances weak endogenous immune responses to these have been identified [3]. Our aim has been to obtain pre-clinical data to support the paediatric development of these agents. We have explored the efficacy of anti-PD-1 and anti-CTLA-4 agents in the syngeneic neuroblastoma models, and demonstrated potent responses, with regression of established tumours and durable disease control, particularly when the two agents are used in combination, or when checkpoint blockade is combined with tumour peptide vaccine. In a spontaneous neuroblastoma model, control of advanced tumour could be achieved by combination of immunomodulatory antibody with low dose cyclophosphamide. Although encouraging, in general the efficacy of immunotherapy agents in pre-clinical models has correlated poorly with clinical responses. More compelling evidence to further the translation of these agents into the paediatric population is therefore provided by the expression of PD-L1 in a range of paediatric cancers. We have demonstrated high levels of membranous expression of PD-L1 in a high proportion of primary human neuroblastoma (72%), alveolar rhabdomyosarcoma (86%), embryonal rhabdomyosarocma (50%), Ewing's sarcoma (57%) and Osteosarcoma (47%). The levels of expression compare favorably with those seen in adult malignancies with proven response to this class of agents. Furthermore, in the 115 tumours examined, increased proportion of CD8+ tumour infiltrating lymphocytes (TILs) correlated with expression of PD-1 expression by the CD8+ cells and patients with PD-L1 positive tumours with a high frequency of TILs had a significantly better survival than patients with PD-L1 negative tumours. This strongly suggests that the PD-1/PD-L1 pathway is active in these tumours, and supports the therapeutic potential of targeting these molecules in childhood cancer.

Although it is hoped that the initial paediatric phase I studies of these agents will provide some signal of response, it may be that this is not seen in this very heavily pre-treated population. Although many of the adult studies have reported responses in patients who have recently received chemotherapy, the degree of immunosuppression may be less than seen in many of the very intensive paediatric treatment protocols. Furthermore, both paediatric pre-clinical studies and adult clinical data suggest that there is significant advantage to be obtained by combinational therapies, and such approaches should be explored in paediatric patients if single agent studies are unsuccessful.

1. Larkin, J., et al., *Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma.* N Engl J Med, 2015. **373**(1): p. 23-34.

2. Rizvi, N.A., et al., *Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer.* Science, 2015. **348**(6230): p. 124-8.
3. Orentas, R.J., et al., *Identification of cell surface proteins as potential immunotherapy targets in 12 pediatric cancers.* Front Oncol, 2012. **2**: p. 194.