Preclinical Development of a Novel Antibody-Drug Conjugate Targeting "Cold" Tumors

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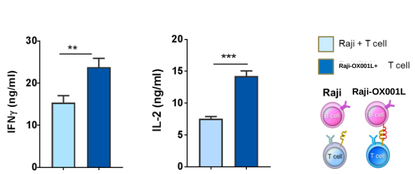
Introduction

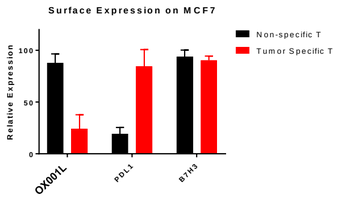
The demonstrated clinical efficacy of immunotherapeutics, which suppress the ability of tumors to evade homeostatic immune system surveillance, validates the promise of checkpoint inhibitors for cancer. Patients whose tumors are inflamed, i.e. posses tumor infiltrating lymphocytes (TILs), and which express the PD-L1 checkpoint ligand are amongst those most likely to benefit from such therapy. Patients whose treatment-refractory tumors do not match this molecular profile require alternative immunotherapies and represent an unmet clinical need. Using our proprietary OGAP® system, we identified a novel immunotherapeutic cancer target, OX001L. IHC studies demonstrated the expression of OX001L across multiple tumor types, with the majority of the OX001L-positive samples staining negative for PD-L1. In NSCLC, OX001L expression was significantly increased in tumors phenotyped as either PD-L1(-) or in the absence of intra-tumoral PD-1(+) T-cell infiltrate compared to inflamed or tumors phenotyped as PD-L1(+). This finding suggests that OX001L may be associated with a "cold" tumor phenotype, which evades cancer immune surveillance. To selectively target OX001L(+) tumor cells, we generated a human OX001L antibody which is cross-reactive to the cynomolgus monkey OX001L. Immunofluorescence microscopy indicated that the antibody-antigen complex exhibits rapid internalization, and that the OX001L antibody, when conjugated to a DNA-alkylating toxin, promoted highly potent *in vitro* cytotoxicity of histotypically distinct cancer cell lines; the OX001L-conjugate also exhibited potent anti-tumor activity *in vivo*. These data demonstrate that the OX001L ADC is an immunotherapeutic molecule active against a molecularly distinct subset of tumor cells refractory to conventional checkpoint-directed PD-1/PD-L1 immunotherapy; thus OX001L ADC may present a unique therapeutic opportunity to address the unmet clinical need of PD-L1(-)/OX001L(+) "cold" tumors, and is currently being advanced towards first-in-human clinical studies.

i. OX001L/OX001R interaction activates T cells



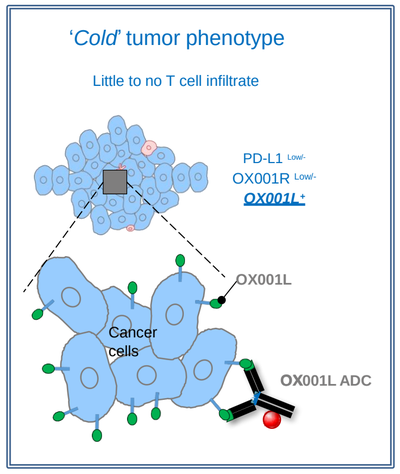
Raji-specific T cell line was co-cultured with OX001L$^+$ or OX001L$^-$ Raji. Cytokine production was measured in co-culture supernatants by ELISA.

ii. OX001L/OX001R and PD-L1/PD1 axes represent orthogonal pathways



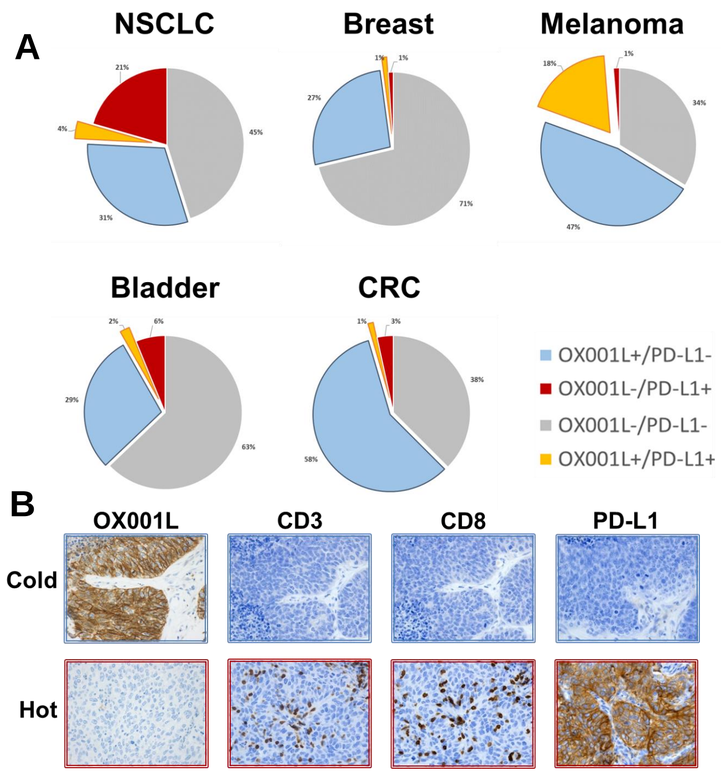
Distinctive mechanisms of regulation: inverse correlation of OX001L and PD-L1 expression

iii. OBT Immune Escape Hypothesis



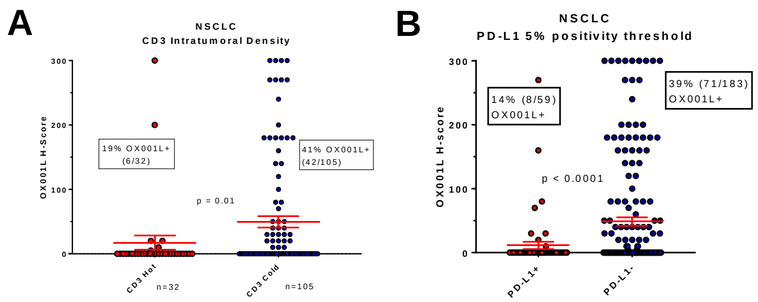
State 1 Homeostasis	OX001L / OX001R interactions mediate tumor killing
	OX001L+ / OX001R+
	CD4+, CD8+, NK+
	T/NK cells kill tumor = Immune surveillance intact
State 2 Immune Escape	T/NK cells present but inactive because missing ligand; tumor progresses
	OX001L downregulated in tumors by T cells
	OX001L- / OX001R+
	PD1/ L1 positive CD4+, CD8+,NK+
	Immune escape due to lack of activation ligand OX001L
State 3 'Cold tumors'	Ligand present on tumor, tumor progresses
	OX001L is NOT downregulated
	OX001L+ / OX001R-
	PD1 / L1 negative CD4 / CD8 negative
	'cold tumors'

iv. OX001L is highly prevalent in 'cold' tumors



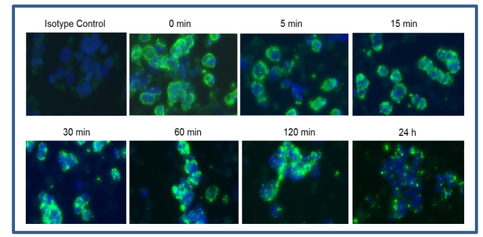
(A) IHC analysis shows OX001L is highly prevalent in multiple 'cold' tumor types. (B) panel shows an example of OX001L staining in a '*hot*' and '*cold*' NSCLC.

v. OX001L exhibits high expression in PD-L1$^-$ tumors



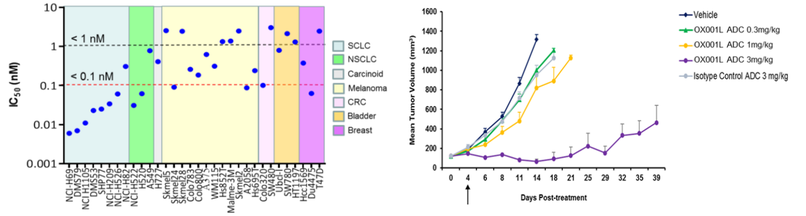
(A) Further analysis reveals OX001L expression is negatively correlated with CD3 (a marker of T cell infiltrate)
(B) OX001L expression is also negatively correlated with expression of PD-L1

vi. Anti-OX001L is rapidly internalised in H526 cells



OX001L+ human SCLC cells (H526) were stained on ice sequentially with anti-OX001L mAb and a fluorescently labelled secondary Ab. Cells were transferred to 37ºC for different time points, fixed, stained with DAPI, and assayed by fluorescence microscopy. Most of the OX001L antigen-antibody complex efficiently trafficked from the plasma membrane to the cytoplasm in 1 hour, with nearly all of the signal appearing as large cytoplasmic aggregates by 2 hours.

vii. Anti-OX001L ADC exhibits highly potent *in vitro* activity and tumor growth inhibition *in vivo*



Cultured different human tumor cell lines were titrated with the OX001L ADC for 3 days. Cell viability was assayed using the Cell Titer Glo assay (Promega) to quantify ATP levels. To generate EC50 values as a measure of cytotoxic potency nonlinear regression analysis was used. The OX001L ADC demonstrated highly potent subnanomolar cytotoxic activity towards many of the OX001L+ tumor cell lines.

Human SCLC cells (H526) were subcutaneously implanted into nude mice. The mice were treated with a single i.v. dose of the anti-OX001L ADC at 0.3, 1 and 3 mg/kg or an isotype control ADC at 3 mg/kg. Treatment of 3mg/kg of the anti-OX001L ADC resulted in maximal tumor growth inhibition and durable partial tumor regressions in 5/8 mice.

Conclusions

- Novel immune escape mechanism: OX001L/OX001R axis
- OX001L highly expressed in PD-L1(-) tumors with low T cell infiltrate
- OX001L(+) "cold" tumors can be potently targeted with OX001L ADC
- OX001L ADC is being advanced towards clinical testing