Harnessing novel immune escape mechanisms for cancer therapeutics: OXAB1 target validation, proof of concept and preclinical development

Livija Deban[1], Wei Cao[2], Yaoyao Fu[2], Angelo Kaplan[2], Rachel L. Dusek[2], Arnima Bisht[2], Sudha Swaminathan[2], San Lin Lou[2], Manal Amoury[2], Bukky Adelegan[1], Joanne Berry[1], James E. Ackroyd[1], Chuck Hannum[2], Robert Boyd[1], Christian Rohlff[1], and Haining Huang[2]

[1]Oxford BioTherapeutics Ltd, Abingdon, United Kingdom; [2]Oxford BioTherapeutics Inc, San Jose, CA

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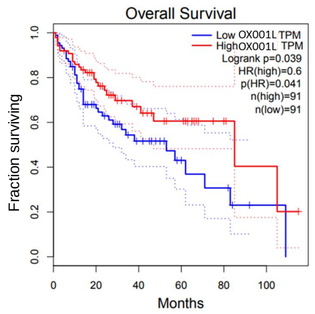
Introduction

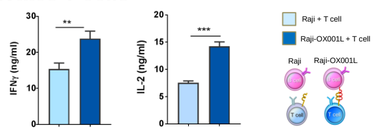
Evasion of the host's immune system by tumor cells is a validated mechanism of tumor establishment and progression; current immunotherapeutics target evasion of homeostatic immune surveillance via modulation of the PD-1/PD-L1 axis. Nevertheless, there remains a significant patient population unresponsive to current immunotherapies. We describe: (i) a novel tumor escape mechanism mediated via the T cell OX001R/OX001L receptor-ligand axis that is orthogonal to the PD-1/PD-L1 axis, and (ii) a therapeutic antibody candidate targeting this axis. The receptor (OX001R) is expressed on activated CD8+ T cells. The ligand (OX001L) is expressed across multiple tumor types. Analysis of overall survival in multiple cancers demonstrated that downregulation of OX001L correlated with worse prognosis. Assay of NSCLC samples revealed the degree of tumor T cell infiltration correlated significantly with OX001L downregulation and PD-L1 upregulation (see poster No. 2772). In addition, assays demonstrated that: (i) the OX001R/OX001L axis in immune cells acts agonistically, (ii) tumor cells downregulate OX001L and upregulate PD-L1 upon T cell engagement, implicating the OX001R/OX001L axis in tumor escape, and (iii) OX001L downregulation requires direct cell-to-cell contact in contrast to the soluble-factor-mediated PD-L1 upregulation, validating orthogonality of these tumor escape axes. Further, to confirm that this axis could be pharmacologically modulated, an anti-OX001R-specific agonist antibody was employed in *ex vivo* 3D tumor explants and an *in vivo* humanized animal model of NSCLC; additivity was observed when the combination of anti-OX001R and anti-PD-1 was assayed. Finally, we have developed OXAB1, an agonistic humanized anti-OX001R antibody, which demonstrated enhanced T cell activation in both *in vitro* and *ex vivo* models relative to other agonistic antibodies. These results validate the role of the OX001R/OX001L axis in evasion of homeostatic immune surveillance, and support the development and clinical translation of our agonistic OXAB1 antibody, either as monotherapy or in combination with other orthogonal immune modulators.

i. Signaling via OX001L/OX001R axis is associated with a better prognosis



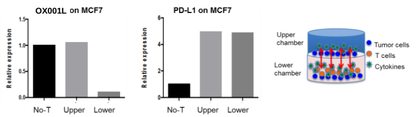
Survival curve for OX001L expression in HCC. Data from TCGA.

ii. OX001L/OX001R interaction activates T cells



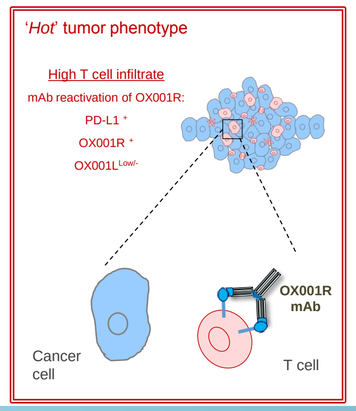
Raji-specific T cell line was co-cultured with OX001L$^+$ or OX001L$^-$ Raji. Cytokine production was measured in co-culture supernatants by ELISA.

iii. OX001L/OX001R and PD-L1/PD-1 axes represent orthogonal pathways



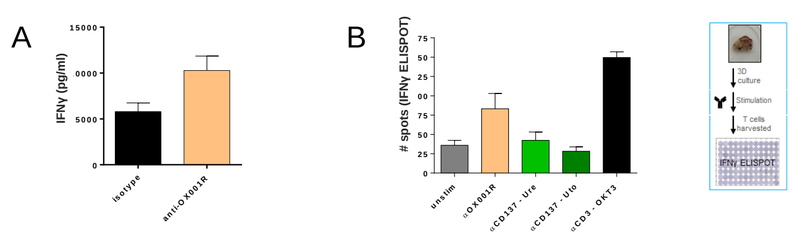
Distinctive mechanisms of regulation: cell-cell contact required for OX001L downregulation; soluble factors mediate PD-L1 upregulation.

iv. OBT Immune Escape Hypothesis



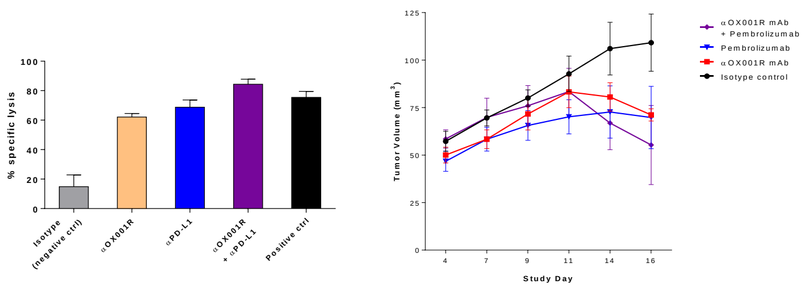
State 1 Homeostasis	OX001L/OX001R interactions mediate tumor killing
	OX001L+/OX001R+
	CD4+, CD8+, NK+
	T/NK cells kill tumor = Immune surveillance intact
State 2 Immune Escape	T/NK cells present but inactive because missing ligand; tumor progresses
	OX001L downregulated in tumors by T cells
	OX001L-/OX001R+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

v. OX001R activates T cells and enhances cytokine release



OKT3-stimulated T cells were treated with commercially available anti-OX001R antibody. (A) Commercially available anti-OX001R antibody enhanced IFNγ production in MLR assay; (B) 3D tumor explants were stimulated for 24h and TIL activation was assessed by IFNγ ELISPOT. Anti-OX001R enhanced cytokine production in both activated T cells and TILs.

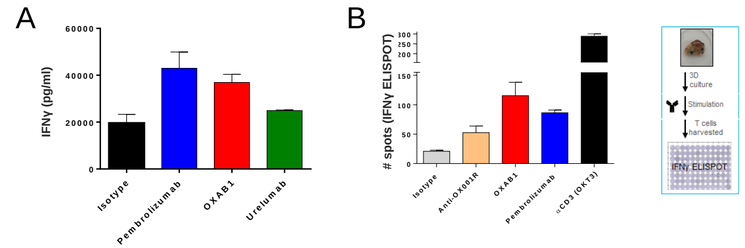
vi. Anti-OX001R induces immune cell mediated cytotoxicity *in vitro* and tumor growth inhibition *in vivo*



Commercially available anti-OX001R antibody enhanced PBMC-mediated cytotoxicity against H322 NSCL cell line comparably to anti-PD-L1 antibody.

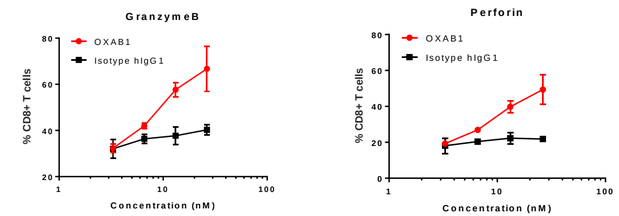
NCG mice were engrafted with human PBMCs and inoculated with the HCC827 NSCLC cell line. Biweekly i.p. administration: 5 mg/kg (pembrolizumab) and 10 mg/kg (Isotype control and anti-OX001R). Single agent anti-OX001R induced 35% tumor growth inhibition (TGI) compared to the control, while addition of pembrolizumab augmented the TGI to 49%.

vii. OXAB1 activates T cells and enhances cytokine release



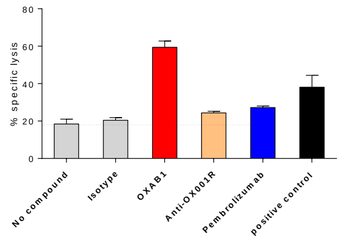
A) OXAB1 antibody enhanced IFNγ production in MLR assay; (B) 3D tumor explants were stimulated for 72h and TIL activation was assessed by IFNγ ELISPOT. OXAB1 enhanced cytokine production in both activated T cells and TILs.

viii. OXAB1 enhances the cytotoxic potential of CD8+ T cells



OKT3-stimulated CD8+ T cells were treated with different concentrations of OXAB1 and granzyme B and perforin production were measured by intracellular flow cytometry. Dose-dependent increase of both cytotoxic proteins shows that OXAB1 enhances T cell cytotoxic potential.

ix. OXAB1 induces immune cell-mediated cytotoxicity



IL-2-stimulated PBMCs were co-cultured with K562 cells and specific cell lysis was measured using RTF. OXAB1 induced immune cell cytotoxicity.

Conclusions

- OX001R activates CD8$^+$ T cells and NK cells
- Anti-OX001R agonist promotes anti-tumor response
- OXAB1 clinical development lead demonstrates superior activity
- Patient selection strategy identified

OBT universal approach to treating cancer patients