Characterisation of novel CD73 antibodies as a therapeutic method of adenosine regulation

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Background

CD73 is a membrane-bound nucleotidase receptor which is frequently overexpressed in the tumour microenvironment and can be found on both tumour and immune cell types. Its function is to catalyse the conversion of adenosine monophosphate (AMP) to adenosine and phosphate and it has been proposed as a therapeutic target in cancer due to the role of adenosine in tumour immune suppression.

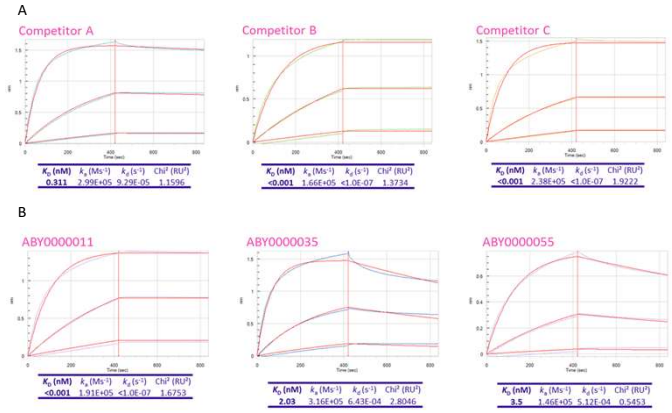
Using multiple approaches, a series of novel CD73 antibodies have been characterised for their therapeutic potential and benchmarked against agents currently undergoing clinical evaluation.

Selection of hit panel

- Hit panel from n-CoDeR® antibody library (BioInvent)
- Phage panning on human and mouse CD73
- 61 unique sequences were converted to full IgG binding human and mouse CD73

Antibodies bind with different affinities

Figure 1

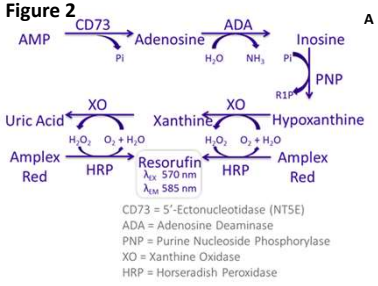


Antibody binding properties to CD73 protein was determined using biolayer interferometry (BLI).
The antibodies tested had comparable association rates (k_a) but variation was detected in the dissociation rates (k_d) resulting in difference in affinity (K_D)

A. Association/dissociation curves of competitor antibodies
B. Association/dissociation curves of selected iOnctura CD73 antibodies with differing K_D

Antibodies directly inhibit enzyme activity

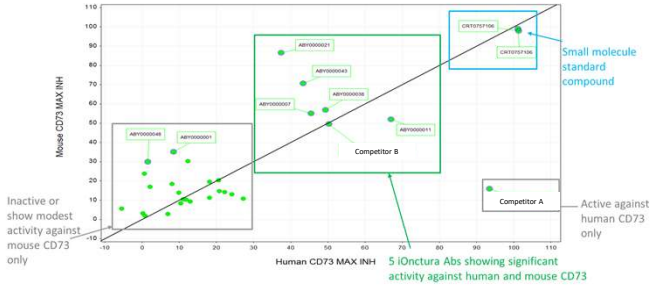
Figure 2



A Assay determines the ability of purified CD73 to catabolize AMP to adenosine. A series of coupling reactions results in the production of Hydrogen peroxide which is then measured using the amplex red system. The assay is read kinetically and initial assay rates are used to calculate percentage inhibition

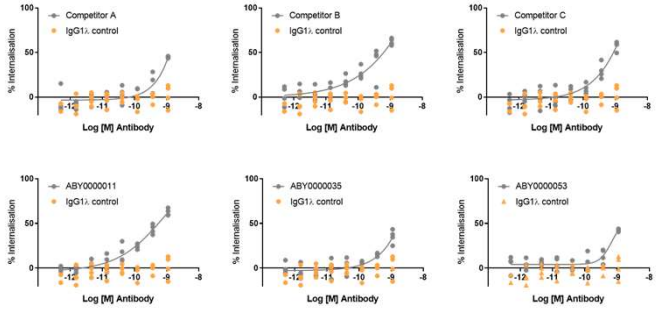
Maximum % inhibition of CD73 antibodies against human and mouse CD73

B



Antibodies induce receptor internalization

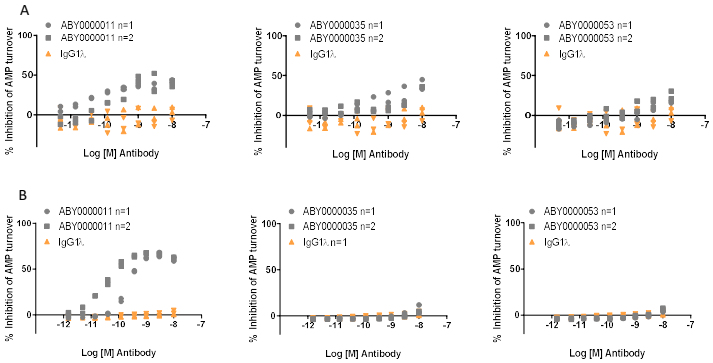
Figure 3



CD73 antibodies were evaluated for their ability to induce receptor internalisation in MDA-MB-231 cells (2,500 cells/well – 384 well plate) using the Fab-ZAP internalisation assay

Antibodies inhibit membrane bound and soluble CD73 activity

Figure 4



A. Membrane Bound: MDA-MB-231 cells (10,000 cells/well - 96well plate) were incubated with antibody for 30min at 37°C followed by 10µM AMP for 30min at 37°C. Reversal of CD73 mediated inhibition of AMP hydrolysis was measured by AMP-Glo

B. Soluble: Human serum from healthy donors was incubated with antibody for 30min at 37°C followed by 10µM AMP for 30min at 37°C. Reversal of CD73 mediated inhibition of AMP hydrolysis was measured by AMP-Glo

Summary

Antibody	% Inhibition of CD73 (Biochemical assay)		% internalisation		% Inhibition of CD73 (Cell assay)	
	Human	Mouse	Human	Mouse	Membrane bound CD73	Soluble CD73
ABY011	63.24	50.18	55.96	70.61	40.72	61.47
Competitor A	91.3	17.75	47.53	6.46	68.21	77.14
Competitor B	42.28	49.07	58.05	70.94	42.00	39.99
Competitor C	20.03	21.29	61.73	7.14	29.9	10.39

- Using multiple approaches, a series of novel, cross-species reactive mAbs have been characterised for mechanism of action of inhibition of CD73
- We demonstrate that CD73 mAbs act by two different mechanisms: direct inhibition of enzyme activity and modulation of CD73 cell surface expression
- Both mechanisms could account for the therapeutic potential to disrupt CD73-mediated adenosine production which in turn will lead to an enhanced anti-tumour immune response
- iOnctura has selected a lead mAb candidate (ABY0000011) from the hit panel for further development as a monotherapy and in combination with a novel small molecule inhibitor for the treatment of cancer

Conflict of Interest Statement: M Deken and Z Johnson are employees and shareholders of iOnctura.

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