P.B2.06.05

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

Gráinne Gernon[1], Suzanne Grooby[1], Louise Tonkin[1], Anja-Christina Bitterwolf[2,3], Lorna Stewart[2], Pritom Shah[1], Zoë Johnson[3], Katherine Ewings[1]

1. Cancer Research UK Therapeutic Discovery Laboratories, Babraham Research Campus, Cambridge, CB22 3AT
2. Cancer Research UK Therapeutic Discovery Labs, London Bioscience Innovation Centre, 2 Royal College Street, London NW1 0NH
3. iOnctura SA, Avenue Sécheron 15, 1202 Geneva, Switzerland

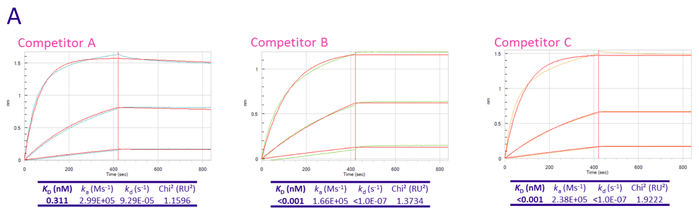
INTRODUCTION

CD73 is a membrane-bound nucleotidase receptor which is frequently overexpressed in the tumour microenvironment and can be found on both tumour and infiltrating immune cells. 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.

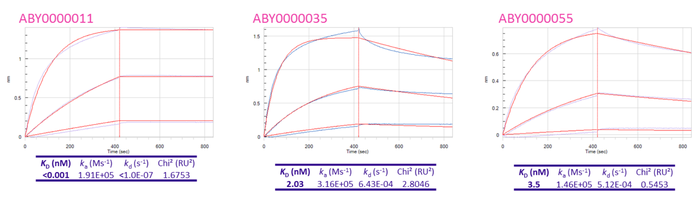
Here using multiple in vitro approaches we have characterised a panel of novel anti-CD73 antibodies to evaluate their therapeutic potential.

1. Antibodies bind to CD73 protein with differing affinities

A



B



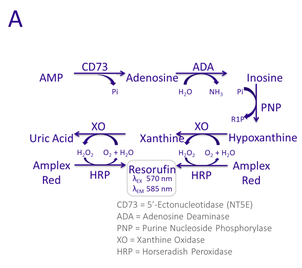
Antibody binding properties to CD73 protein was determined using biolayer interferometry (BLI)[1]. The antibodies tested had comparable association rates (k_a) but variation was detected in the dissociation rates (k_d) resulting in difference in affinity (K_D) A. Association/dissociation curves of competitor antibodies B. Association/dissociation curves of three internal CD73 antibodies with differing K_D.

References

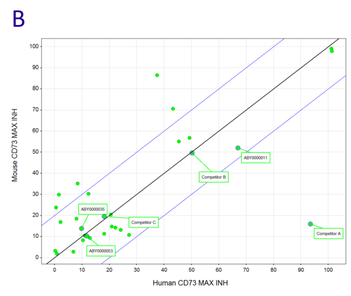
1.http://www.fortebio.com/interactions/Spring_2012/page5.html
2.https://www.essenbioscience.com/media/uploads/files/8000-0572-A00--FabFluor_Red_Ab_Label_Reagent_App_note.pdf

2. CD73 antibodies show direct inhibition of enzyme activity

A

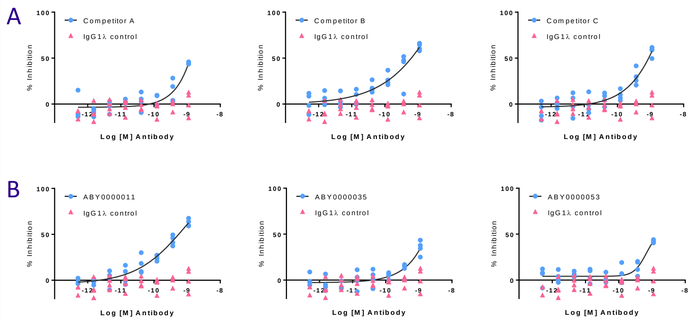


B



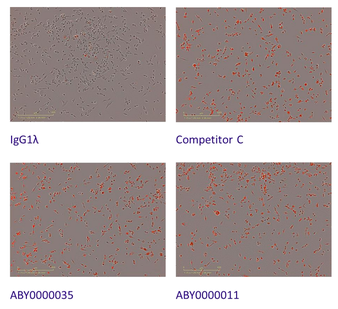
A. Assay determines the ability of purified CD73 to catabolize AMP to adenosine. A series of coupling reactions results in the production of Hydrogen peroxide which is then measured using the amplex red system. The assay is read kinetically and initial assay rates are used to calculate percentage inhibition.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

3. CD73 antibodies induce receptor internalisation



CD73 antibodies were evaluated for their ability to induce receptor internalisation in MDA-MB-231 and 4T1 cells (2,500 cells/well – 384 well plate) using the Fab-ZAP internalisation assay. A. % inhibition of competitor antibodies B. % inhibition of top internal CD73 antibodies (data representative of n=2)

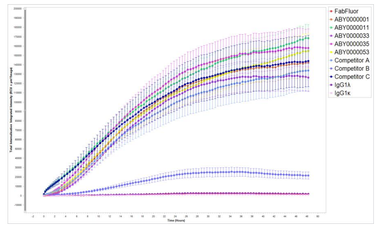
A



C

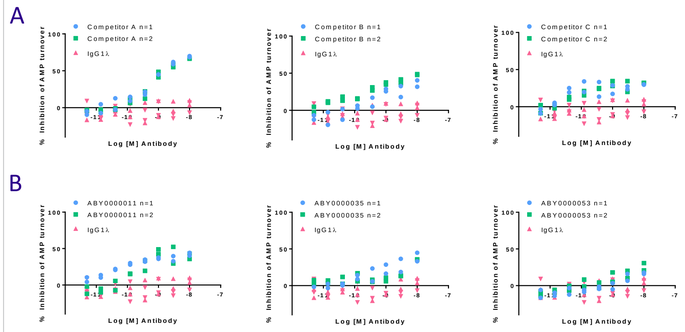
Aby 0.4µg/ml	AUC
ABY0000035	3514916
ABY0000011	3268551
Competitor A	2932633
ABY0000053	2795065
ABY0000001	2638716
Competitor C	2536839
ABY0000033	2493891
ABY0000012	2170492
ABY0000005	1924279
ABY0000014	1408848
ABY0000060	1389991
ABY0000048	672214
ABY0000038	650455.4
Competitor B	419636.5
ABY0000055	394397.3
ABY0000010	337901.1
ABY0000015	303248.4
ABY0000023	96456.26
ABY0000021	63512.35
IgG1κ	53895.27
ABY0000040	51214.29
ABY0000043	49767.53
ABY0000006	48884.66
ABY0000007	46584.77
IgG1λ	31293.25
FabFluor alone	22562.9

B



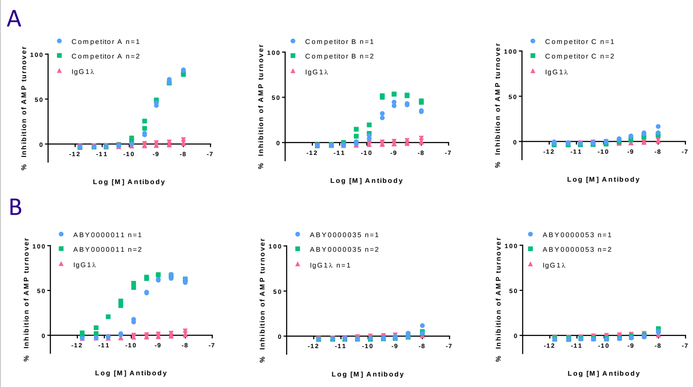
Receptor internalisation was measured using IncuCyte® Human FabFluor-pH Red Antibody Labelling Reagent[2] on IncuCyte®S3 A. MDA-MB-231 cells were incubated with 0.4ug/ml CD73 antibody with FabFluor reagent for 24hrs (1:0.5 molar ratio) B. Graph illustrating top hits plus competitor antibodies. C. Images were assessed for red fluorescent signal, quantified using IncuCyte®software and ranked for internalisation by AUC analysis.

4. CD73 antibodies show inhibition of membrane-bound CD73-mediated hydrolysis of AMP



MDA-MB-231 cells (10,000 cells/well - 96well plate) were incubated with antibody for 30min at 37ºC followed by 10µM AMP for 30min at 37ºC. Reversal of CD73 mediated inhibition of AMP hydrolysis was measured by AMP-Glo. A. Competitor antibodies B. Top internal CD73 antibodies.

5. CD73 antibodies show inhibition of soluble CD73-mediated hydrolysis of AMP



Human serum from healthy donors (pooled) was incubated with antibody for 30min at 37ºC followed by 10µM AMP for 30min at 37ºC. Reversal of CD73 mediated inhibition of AMP hydrolysis was measured by AMP-Glo. A. Competitor antibodies B. Top internal CD73 antibodies.

CONCLUSIONS

We demonstrate, that amongst our panel of antibodies, candidates which inhibit CD73 function by two different mechanisms, direct inhibition of enzyme activity and modulation of cell surface expression; both of which have therapeutic potential to disrupt CD73-mediated adenosine production and therefore reduce anti-tumour immune responses.

Several antibodies from this panel will be advanced into late-stage preclinical development to identify a clinical candidate.

Acknowledgements
Antibodies were generated by BioInvent

iOnctura

CANCER RESEARCH UK | THERAPEUTIC DISCOVERY LABORATORIES