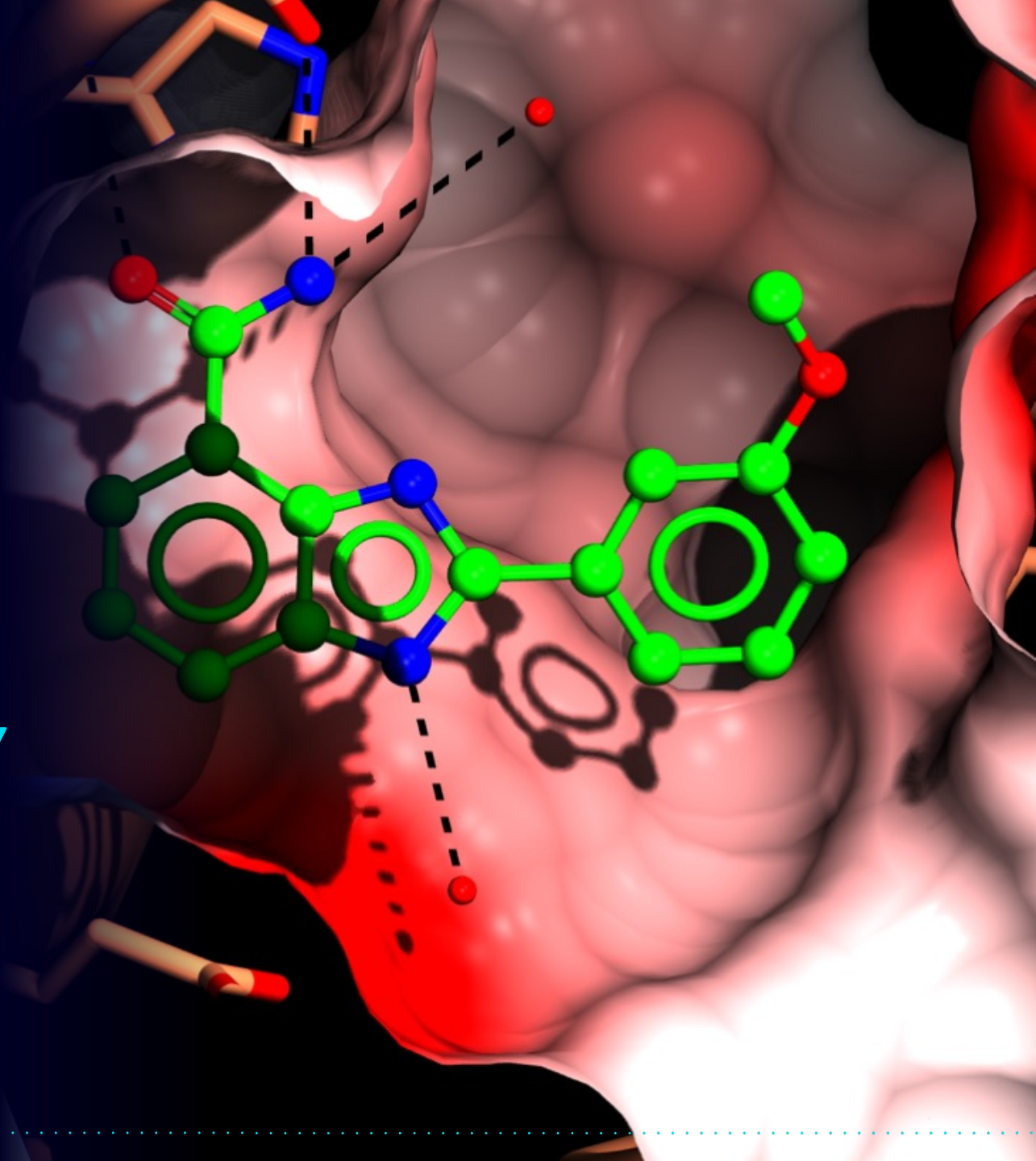
CANCER RESEARCH HORIZONS

FURTHER FASTER TOGETHER

LICENSING OPPORTUNITY:

FIRST IN CLASS ARGINASE 2 INHIBITORY ANTIBODY

Sept 2021



OPPORTUNITY OVERVIEW

- Collaboration between CRUK AstraZeneca Alliance Lab (AAL) (https://www.cancerresearchuk.org/funding-for-researchers/our-research-infrastructure/cruk-astrazeneca-antibody-alliance-laboratory) and Prof Vincenzo Cerundolo's lab at Oxford University
- Developed first-in-class inhibitory antibodies that potently and selectively inhibit arginase 2 (Arg2), using phage display technology. Antibodies are fully human with favourable pharmacokinetics. Published May 2020 in PNAS (https://www.pnas.org/content/pnas/early/2020/07/01/1919565117.full.pdf)
- Lead molecule C0021061 has been assessed in vivo and chosen for further development (see subsequent slides and July 2020 publication in mAb: https://www.tandfonline.com/doi/full/10.1080/19420862.2020.1801230)
- Potential indications include oncology, COPD, cystic fibrosis and atherosclerosis
- International Patent Application No. PCT/EP2020/073579, filed 21 August 2020

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DEVELOPMENT OF THE LEAD MOLECULE C0021061

ARGINASES IN HEALTH AND DISEASE

- Arginases are metabolic enzymes responsible for arginine metabolism. They play a fundamental role in the urea cycle, which provides protection against excess ammonia, while its metabolites are needed for cell proliferation, collagen formation, and other physiological functions.
- It has been noted that the two isoforms, ARG1 and ARG2, can have effects in different disease settings. Their dysregulation has been linked to disorders associated with inflammation and immunity, including:
  - Cancer
  - Cardiovascular and pulmonary diseases
  - Renal diseases
  - Response to infectious agents
  - Neurovascular diseases

Table 1. *Pathological conditions associated with detrimental effects of increases in arginase 1 and 2 expression and/or beneficial effects of arginase inhibitor or suppressor treatments*

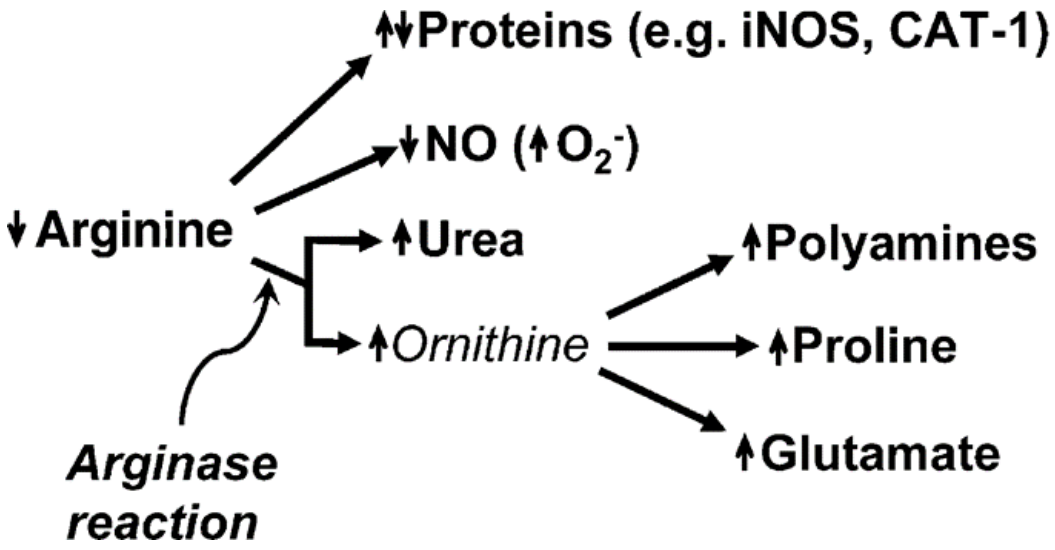
Pathological Condition	Reference Nos.
Hypertension	7, 16, 195, 213, 214
Pulmonary artery hypertension	28, 34, 35, 38, 75, 98, 99, 225, 234, 235
Sickle cell disease	8, 205
Diabetic vascular disease	5, 13, 15, 17, 55, 77, 176, 178, 198, 210
Atherosclerosis	156, 157, 182, 184
Myocardial I/R injury	72, 78, 101, 217, 253
Aging/cellular senescence	14, 111, 188, 233
Erectile dysfunction	21, 39, 43, 112, 119, 190, 213, 216
Diabetic nephropathy	143, 243, 244
Hypertensive nephropathy	129
Cancer	65, 93, 136, 141, 174, 241
Ischemic stroke	162, 167
Traumatic brain injury	18
Alzheimer's disease	31, 37, 81, 90, 124
Multiple sclerosis	25, 76, 125, 126
Retinal disease	55, 151, 158, 201, 207, 252

Caldwell et al. Physiol Rev. 2018

INHIBITING ARGINASE-2 – PROJECT HYPOTHESIS

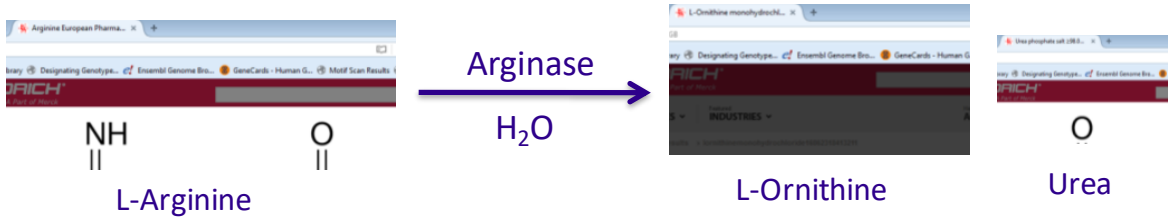
- Extracellular arginase-2 is upregulated in various cancers causing reduced extracellular arginine concentration
- A reduced extracellular arginine concentration can cause reduced T-cell mediated anti tumour responses

Hypothesis: An ARG2-specific inhibitory monoclonal antibody would restore anti-tumour immunity in cancer patients and improve overall survival

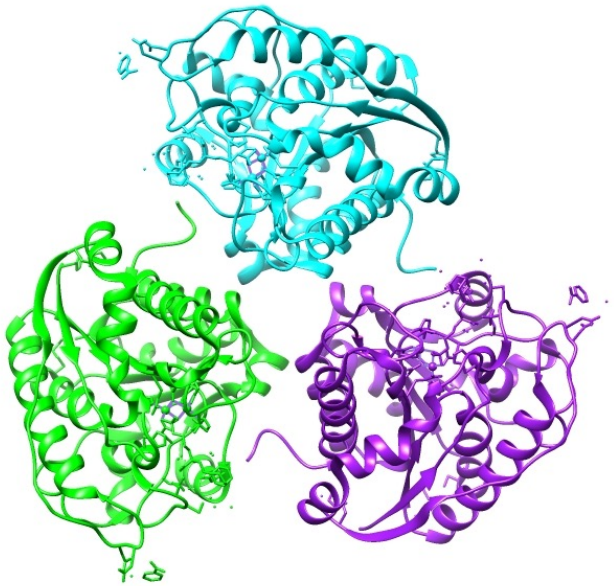


ARGINASES

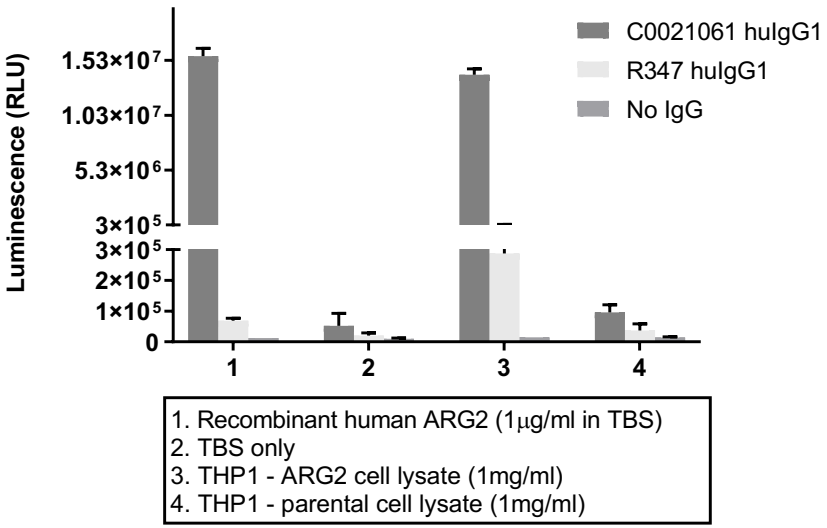
- Arginase-1 and arginase-2 enzymes catalyse the conversion of arginine to ornithine and urea. They have 60% identity.

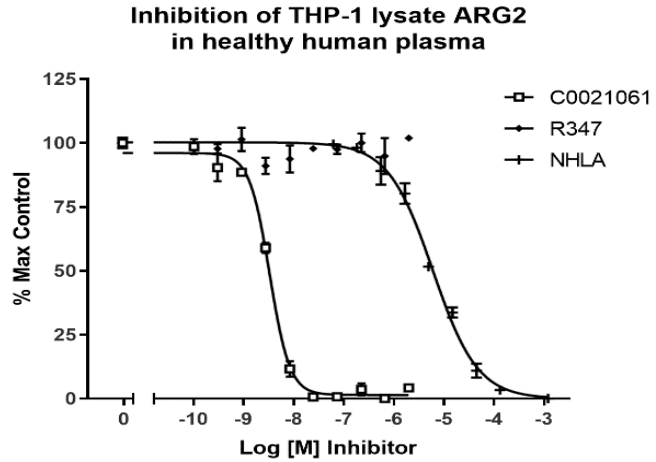


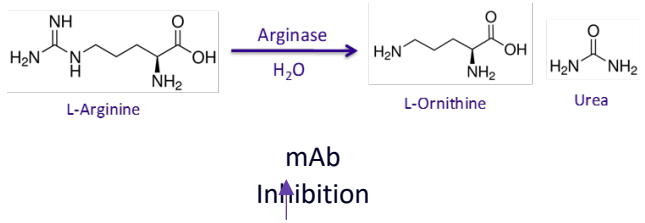
- Arginase-1 is a cytosolic enzyme abundantly expressed in the liver.
- Arginase-2 is a mitochondrial enzyme predominantly expressed in the kidney.
- Both enzymes are homotrimeric with manganese ions at the catalytic site.



C0021061 SHOWS STRONG AND SPECIFIC BINDING TO HUMAN ARG2 AND INHIBITS ENZYMATIC ACTIVITY





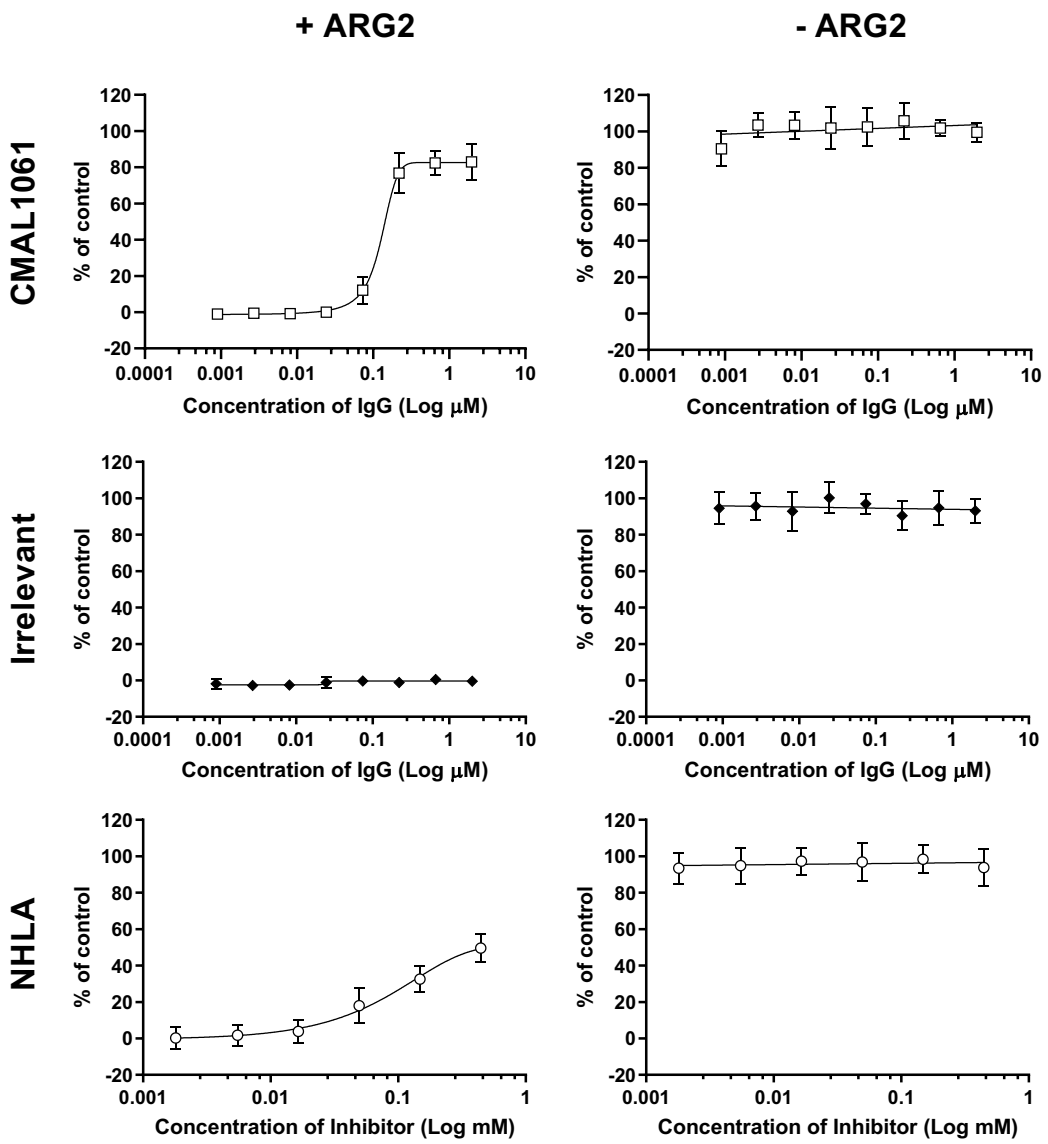


Inhibition of THP-1 (human monocytic cell line derived from acute monocytic leukemia patient (AML)) derived human ARG2 activity by antibodies in the presence of human plasma. C0021061 inhibited the activity of THP-1 lysate derived human trimeric ARG2, with an IC_{50} of approximately 3 nM. The isotype control antibody R347 did not inhibit the activity of THP-1 lysate derived human trimeric ARG2 as expected

Chan, DTY *et al* (2020) PNAS, 117(29):16949-16960.

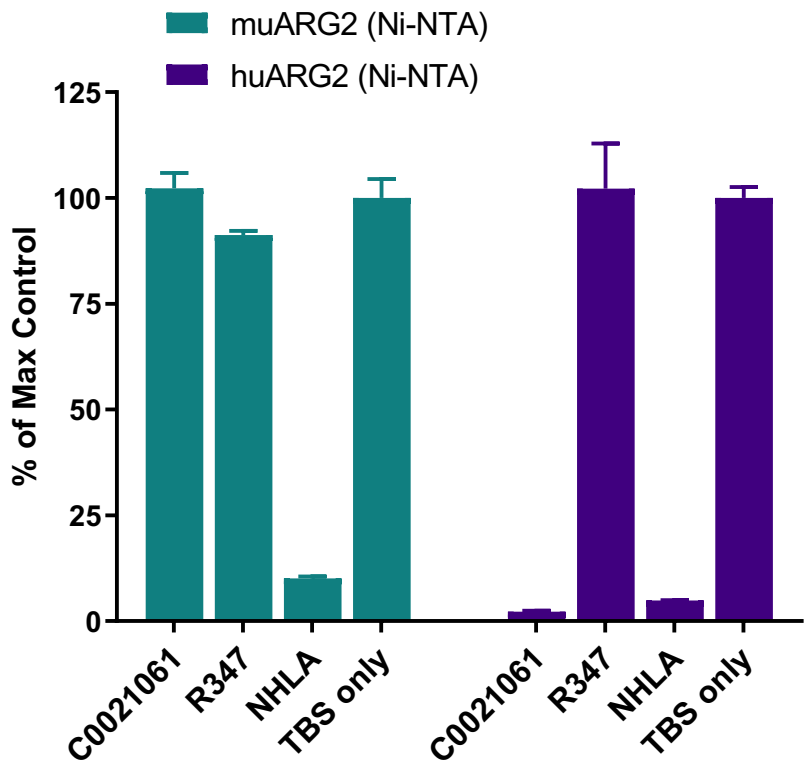
C0021061 RESTORES T CELL PROLIFERATION *IN VITRO*

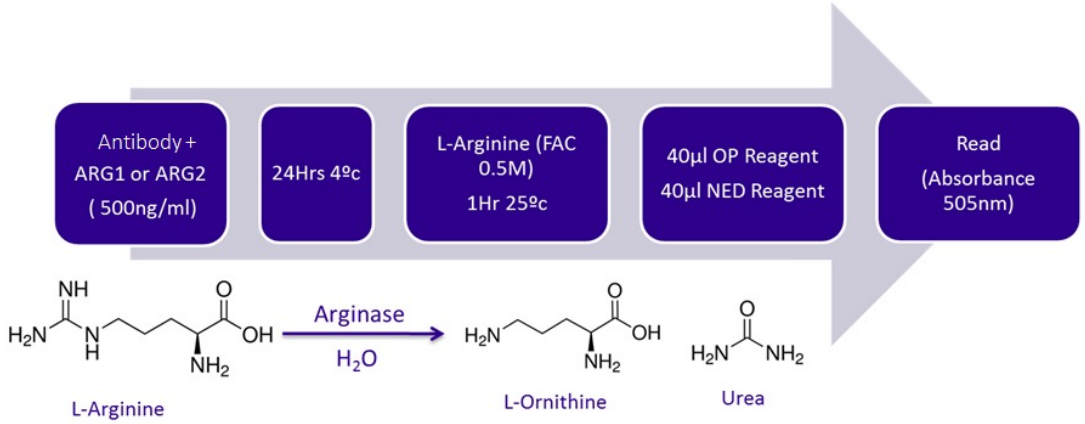
- C0021061 huIgG1 can relieve ARG2-mediated suppression of T cell proliferation *in vitro*, whereas R347 as an isotype control showed no such effect. T cells isolated from PBMCs were incubated in the absence / presence of recombinant trimeric ARG2 (15 µg/ml) with a titration of the antibody.



Austin M., *et al* (2020) MABS, Vol12, No.1; e1801230

C0021061 IS SPECIFIC FOR HUMAN ARG2





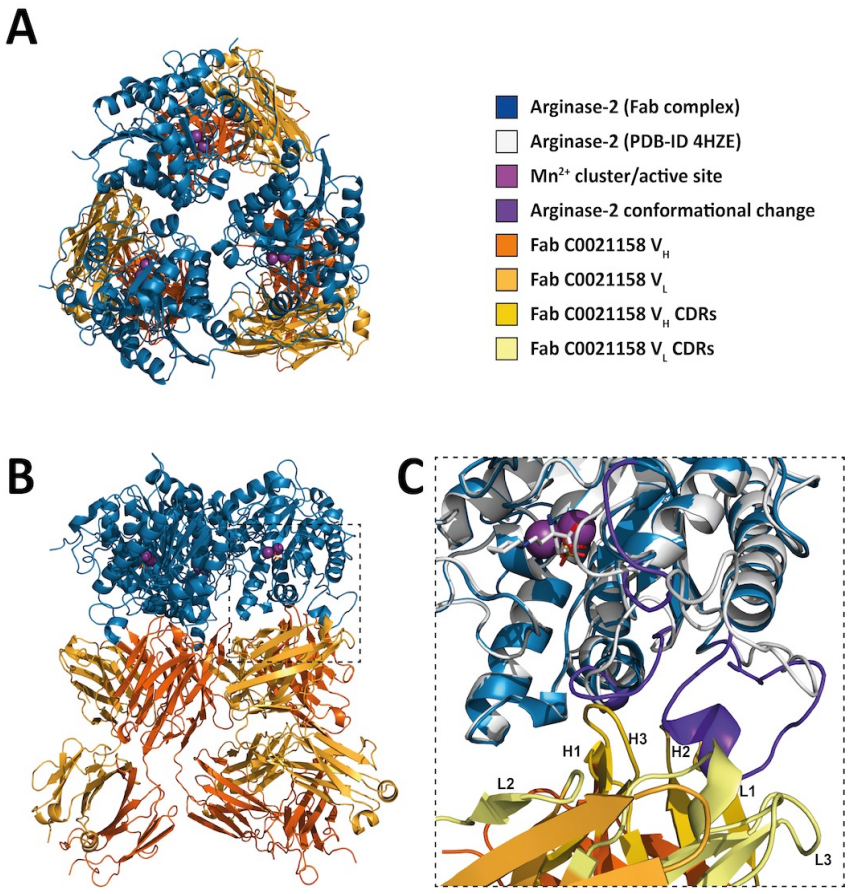
Enzyme inhibition assay showing the inhibition activity of C0021061 against human (hu) or murine (mu) ARG2. NHLA and R347 were included as positive and negative controls respectively.

C0021061 HAS FAVOURABLE KINETICS

- ANTIBODIES C0021061 AND ISOTYPE CONTROL R347 WERE DOSED IN C57/BL6 MICE I.P. AT 10 MG/KG AND SERUM TAKEN AT 2H, 4 DAYS, 7 DAYS AND 14 DAYS.

PK parameters	C0021061	R347
Clearance (ml/hr/kg)	0.352	0.228
Half life (hr)	274	391
Plasma Volume (Vcentral) L/kg	0.139	0.128
Peripheral Volume (Vperipheral) L/kg	Not reported	Not reported

X-RAY CRYSTALLOGRAPHY OF A CLOSELY RELATED ANTIBODY REVEALS A NOVEL ALLOSTERIC MECHANISM OF ARG2 INHIBITION

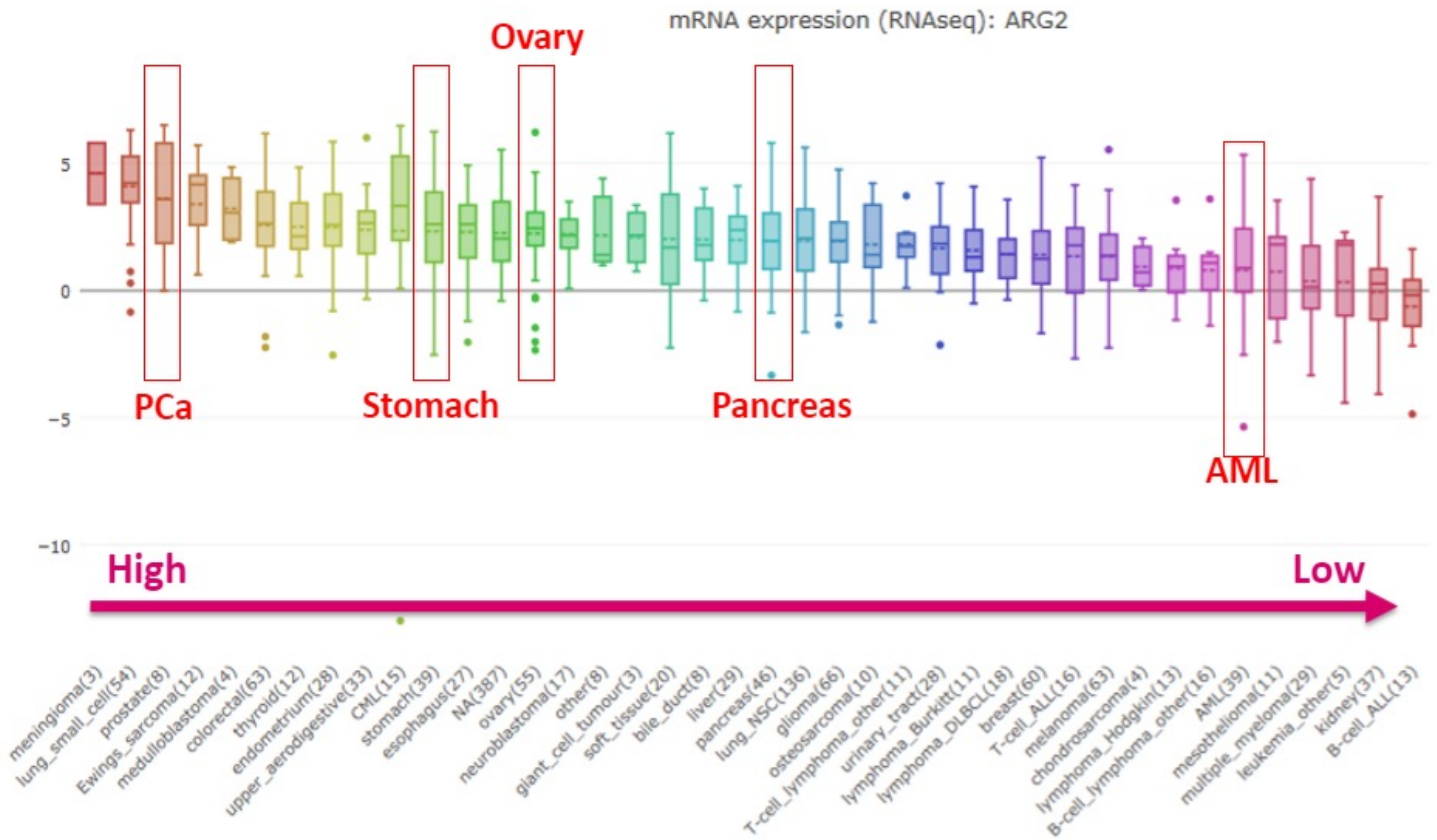


- **ARG2 trimer (blue) bound to C0021158 Fab (VH/CH in orange and VL/CL in light orange).** (A) 'Top down' and (B) 'side' view. In the active sites, manganese atoms are shown as purple spheres and a sulphate ion as sticks. (C) Close-up of the boxed region in B. Free ARG2 (PDB-ID 4HZE, light grey 36) was superimposed on C0021158 Fab-bound ARG2 (excluding the regions of conformational change, shown in dark purple). The CDRs on the Fab are shown in yellow (VH) and light yellow (VL). Significant external changes on the outside of ARG2 distal from the active site lead to subtle internal changes within the active site which prevent access/binding of L-arginine.
- Performed in collaboration with Prof Mark Carr, University of Leicester

Austin M., *et al* (2020) MABS, Vol12, No.1; e1801230

POTENTIAL AREAS FOR ONCOLOGY DEVELOPMENT

- Broad Institute Cancer Cell Line Encyclopedia analysis shows broad range of expression at mRNA level in human cell lines



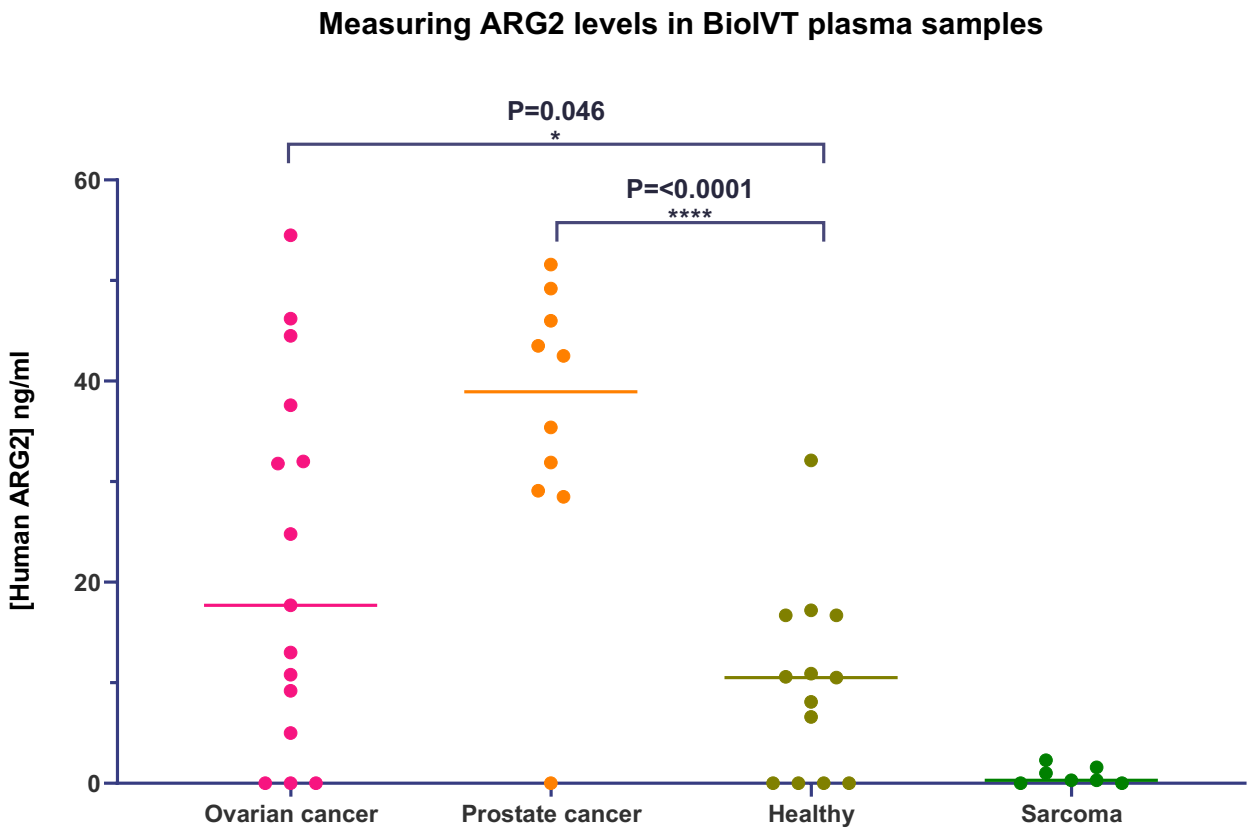
DETECTION OF EXTRACELLULAR ARG2 PROTEIN

- IN-HOUSE ANALYSIS OF CONDITIONED MEDIA FROM SEVERAL CANCER CELL LINES IDENTIFIED SOME CELL LINES PRODUCING EXTRACELLULAR ARG2 PROTEIN

S. No.	Cell Line	Tissue of origin	ARG2 protein in CCM
1	A673	Sarcoma	+
2	HT-1080	Sarcoma	-
3	Saos-2	Sarcoma	+
4	U2-OS	Sarcoma	-
5	SKOV-3	Ovarian	-
6	OVCAR-3	Ovarian	+/-
7	A2780-Parental	Ovarian	+
8	A2780-Cis	Ovarian	+
9	A2780-ADR	Ovarian	+
10	DU145	Prostate	-
11	LNCaP	Prostate	+
12	PC3	Prostate	-
13	PNT2*	Prostate	-
14	AGS	Gastric	+
15	HuTu-80	Gastric	+
16	KATO-III	Gastric	+
17	NCI-N87	Gastric	-
18	SNU-1	Gastric	-
19	SNU-5	Gastric	+/-
20	SNU-16	Gastric	+
21	DX3	Skin	+/-
22	HPAF-II	Pancreatic	+/-
23	MDA-MB-231	Breast	-
24	K562	H & L (CML)	-
25	KMS11	H & L (Multiple Myeloma)	+
26	HL-60	H & L (AML)	+/-

ARG2 PROTEIN CAN BE DETECTED IN SOME PATIENT PLASMA SAMPLES

- In-house quantification of ARG2 protein in serum/ plasma samples from prostate, sarcoma and ovarian cancer patients. ARG2 was quantified using ELISA.

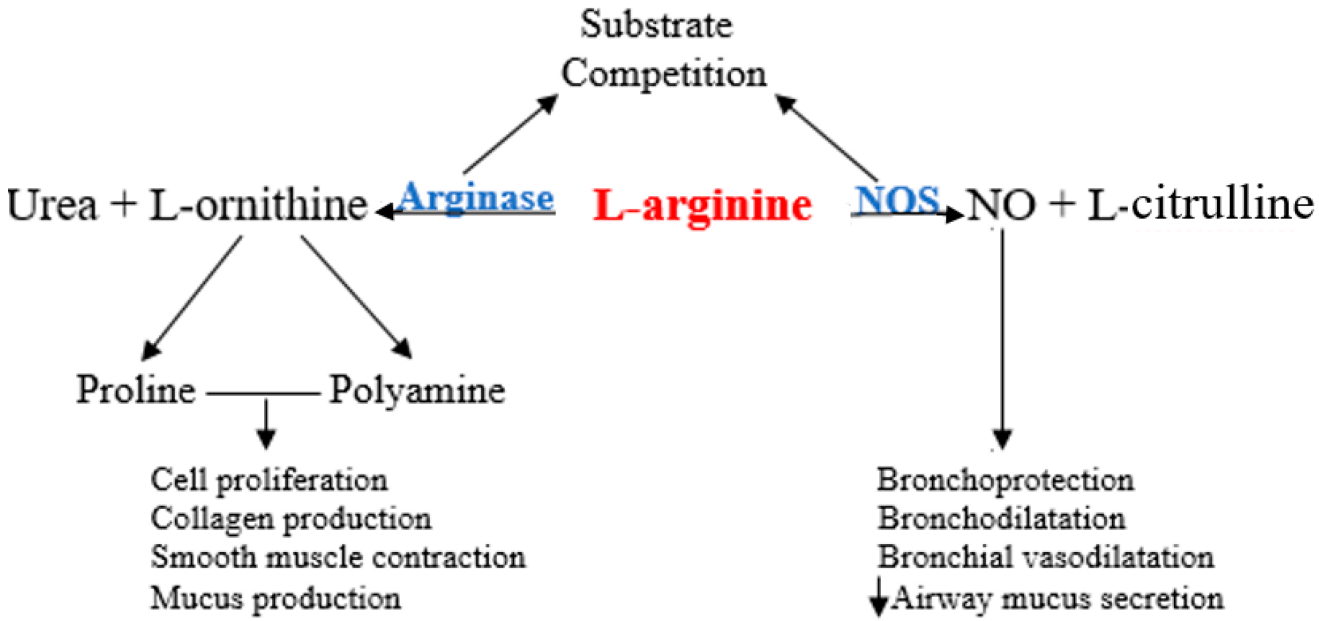


POTENTIAL DEVELOPMENT OF C0021061 OUTSIDE ONCOLOGY SPACE

- Arginases have been implicated in disease progression of several lung conditions and other disease areas including:
  - Cystic Fibrosis (CF)
  - COPD
  - Asthma
  - Atherosclerosis
  - Erectile dysfunction
  - Renal nephropathies
  - Colitis
  - Multiple sclerosis
  - Sickle cell disease / thalassaemia

POTENTIAL FOR ARGINASE INHIBITORS IN CYSTIC FIBROSIS

- CF patients have excessive arginase activity in the lungs (sputum) resulting in low nitric oxide levels
- NO involved in smooth muscle relaxation and bronchodilation
- Reduced NO impairs anti-microbial immune response
- Arginase and NOS compete for arginine



J. Clin. Med. **2019**, *8*(11), 1809; https://doi.org/10.3390/jcm8111809

ARGINASE MODULATORS IN DEVELOPMENT

Company	Drug	MoA	Latest stage of development	Study designs
Calithera/Incyte	INCB001158	NCE (inhibits ARG1 and ARG2)	Phase1/2	Combo with Keytruda in solid tumours Combo with chemo is solid tumours including ovarian, gastric
Calithera	CB280	NCE (inhibits ARG1 and ARG2)		Cystic Fibrosis Ph1b
OncoArendi	OAT-1746 (OATD-02)	NCE (inhibits ARG1 and ARG2)	Pre-clin. Expect Phase I Q4 2020	n/a
Aeglea	pegzilarginase	Modified pegylated arginase	Phase 1/2	combo with Keytruda in SCLC patients (also single agent in patients with hyperargininemia)
Polaris Pharma	ADI-PEG	Arginine depletion via inhibition of arginine deiminase	Multiple completed trials, including Ph3 monotherapy in HCC	Phase I combo with Keytruda Ph2 combo with FOLFOX in GI Ph2/3 mesothelioma in combo with pem/cis
BCT International	BCT-100	Pegylated arginase I	Phase I	Paediatric patients Second study to optimise dose in arginine auxotrophic solid tumours

SUMMARY AND NEXT STEPS

- AAL have developed a lead optimised antibody ARG2 inhibitor which is:
  - First-in-class
  - Selective for ARG2 vs ARG1 therefore differentiated from competitor NCE's
  - Active in restoring T cell proliferation in vitro

- PCT filed 2019, strong IP position

- Available for licensing

CANCER RESEARCH HORIZONS

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THANK YOU

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