

# Identification of a novel non-brain penetrant A<sub>2A</sub>R inhibitor and proof-of-concept of CD73 and A<sub>2A</sub>R/CD73 small-molecule inhibitors for cancer immunotherapy

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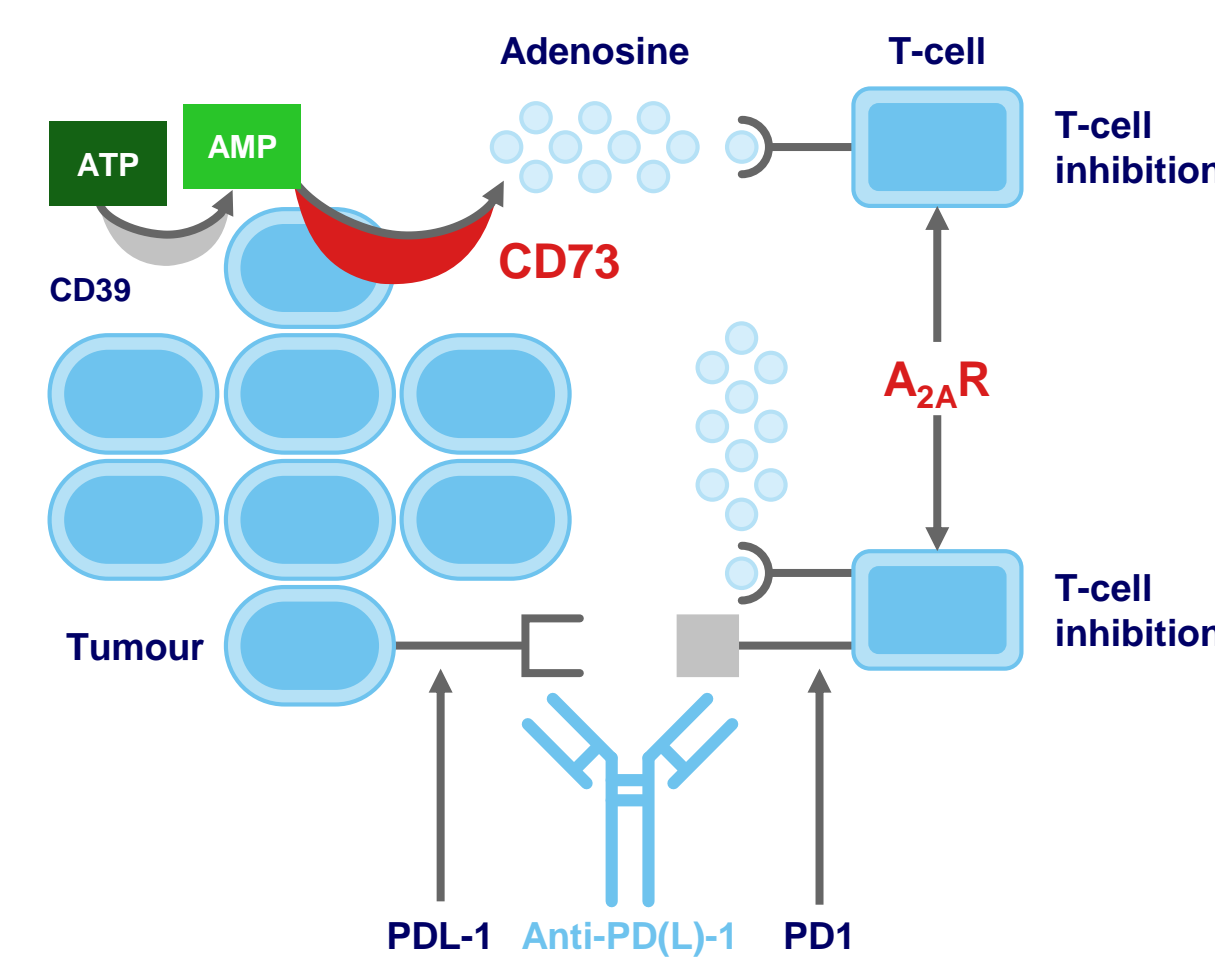
## Overview

<b>Project concept</b>	● Partnership to discover adenosinergic molecules for immuno-oncology therapeutics established between Exscientia and Evotec
<b>Strategy</b>	● Create patentable high quality assets and value in immuno-oncology
<b>Target class</b>	● Specific or bi-specific molecules
<b>Project status</b>	● pre-development candidate identified for A <sub>2A</sub> R inhibitor: Best-in-class ● Lead candidate identified for CD73: First-in-class
<b>Primary indication</b>	● Combination with immune checkpoint therapies for non responder patients
<b>Administration</b>	● Oral administration
<b>Biomarker</b>	● Patient stratification: CD73 positive tumour ● PD biomarkers and biomarker of activity to detect CD73 inhibition and A <sub>2A</sub> R inhibition identified

## Strategy to develop small molecules inhibiting A<sub>2A</sub>R and CD73

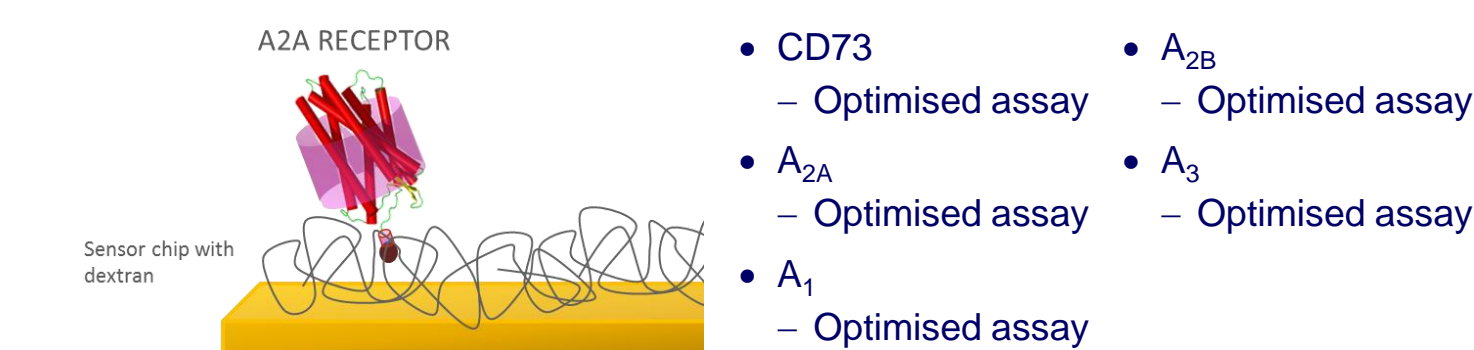
### Effects expected from the A<sub>2A</sub>R and CD73 small molecule inhibitors

- Overcoming immunosuppression
  - Enhanced T lymphocyte and NK cell activity
  - Decreased tumour cell proliferation
- For CD73 inhibition
  - Inhibiting circulating myeloid derived suppressor cells
  - Inhibiting tumour angiogenesis
  - Inducing blood vessel normalization
  - Improving blood vessel extravasation



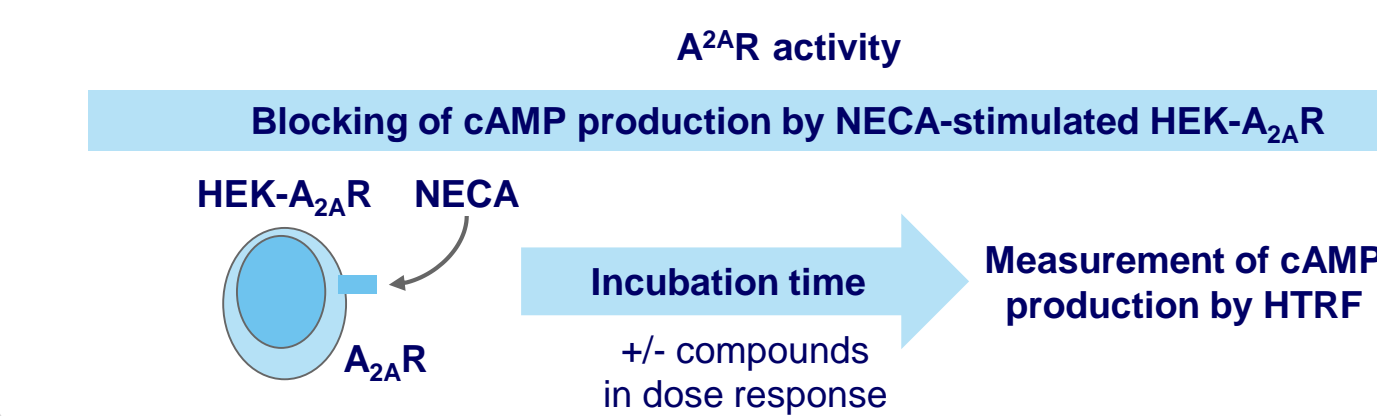
## In vitro assays developed at Evotec/Exscientia

### SPR screening assay with A<sub>2A</sub>R bound to the CHIP allows evaluation of fragments or compounds

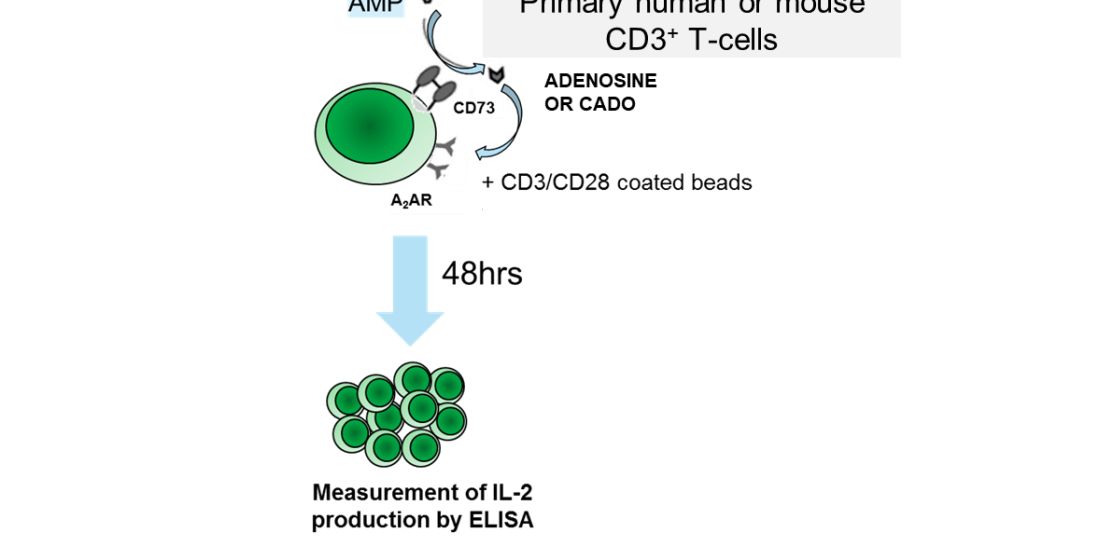


- CD73
  - Optimised assay
- A<sub>2A</sub>
  - Optimised assay
- A<sub>1</sub>
  - Optimised assay
- A<sub>2B</sub>
  - Optimised assay
- A<sub>3</sub>
  - Optimised assay

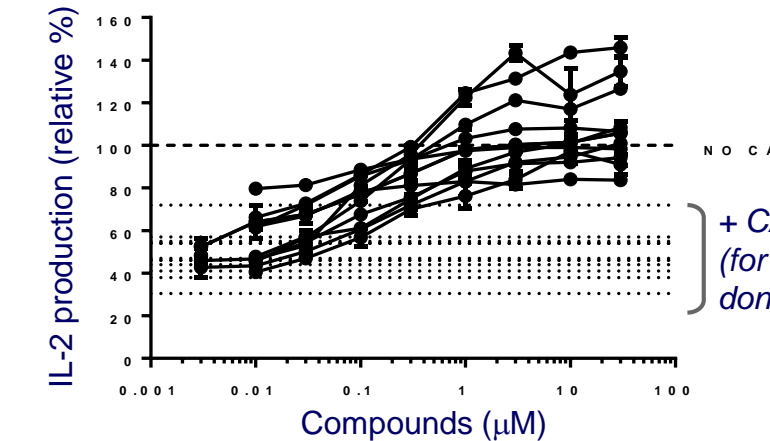
### HEK A<sub>2A</sub>R assay



### Ex vivo functional assay on human or mouse CD3<sup>+</sup> T cells



### Example of A<sub>2A</sub>R inhibitor on recovery of IL2 production induced by CADO



## EVOEXS21546 is a specific, non brain penetrant A<sub>2A</sub>R antagonist

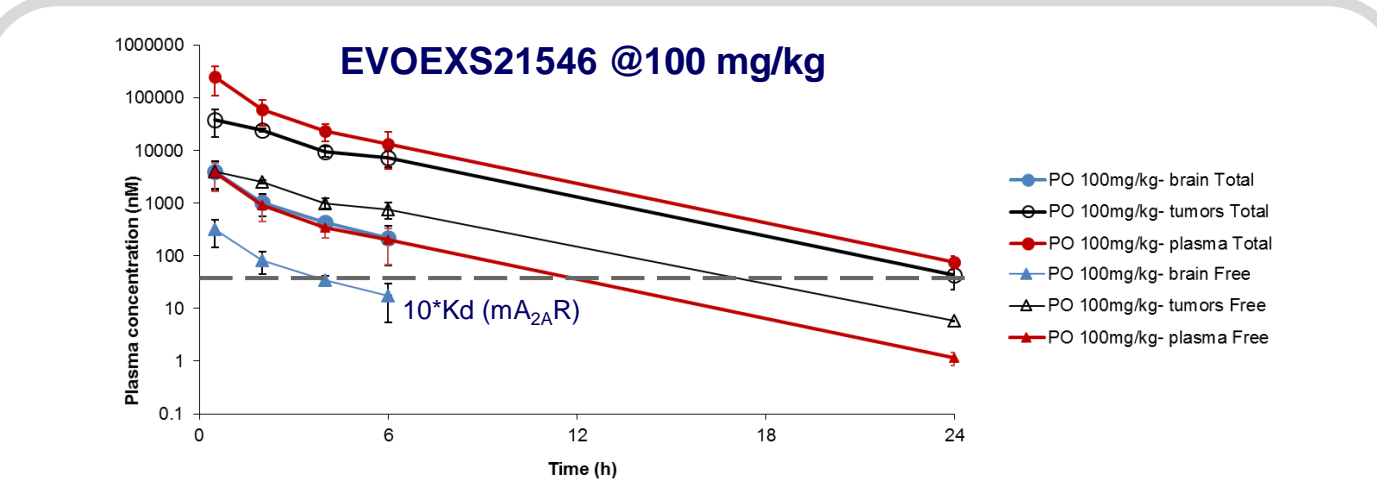
Name	EVOEXS21546
Brain penetrant	NO
Human SPR hA <sub>2A</sub> KD (nM)	6
Mouse SPR A <sub>2A</sub> KD (nM)	7
Human SPR A <sub>2B</sub> A <sub>1</sub> /A <sub>3</sub> KD (nM)	1500 / 3130 / 35790
HEK-Human A <sub>2A</sub> IC <sub>50</sub> (nM) internal/eurofins	37 / 24
Human A <sub>2A</sub> functional – EC <sub>50</sub> (nM)	526
Mouse A <sub>2A</sub> functional – EC <sub>50</sub> (nM)	229
Cl <sub>int,app</sub> (H, µL/min/mg)	10
Caco-2 A→B (10 <sup>-5</sup> cm/sec.) (Efflux ratio)	6.4 (1.7)
LogD (pH 7.4)	1.5
Sol. pH 1 / 7.4 (µg/ml)	235 / 12
Mics Cl <sub>int,app</sub> (µL/min/mg): H/R/M	12 / 25 / 27
Heps Cl <sub>int,app</sub> (µL/min/10e <sup>6</sup> cells): H/R/M	4 / 14 / 32
PPB % bound: H/R/M	97.0 / 97.9 / 98.3

### EVOEXS21546 profile

- Off-target profile @ 10 µM (eurofins)
  - 47 GPCRs evaluated: only 5HT<sub>2</sub> at 50% (plus A<sub>3</sub>: 80% & A<sub>1</sub>: 100%)
  - 5 Ion channels evaluated: no alert
  - 3 transporters evaluated: no alert
- Kinase profile @ 1 µM (Eurofins)
  - 174 kinases evaluated: no alert
  - Highest activity seen on GRK2 (h): 23%
- No cytotoxicity
  - Yoyo1 HEK wt, 10 µM: 1%
- No Cyp inhibition
  - 1A2, 2C19, 2C9 & 2D6> 50 µM
  - 3A4 = 21 µM
- No hERG alert
  - IC<sub>50</sub> > 30 µM
- Ames negative (up to 125 µg/mL)
  - with or without S9 metabolic activation

## EVOEXS21546 is a pre-development candidate

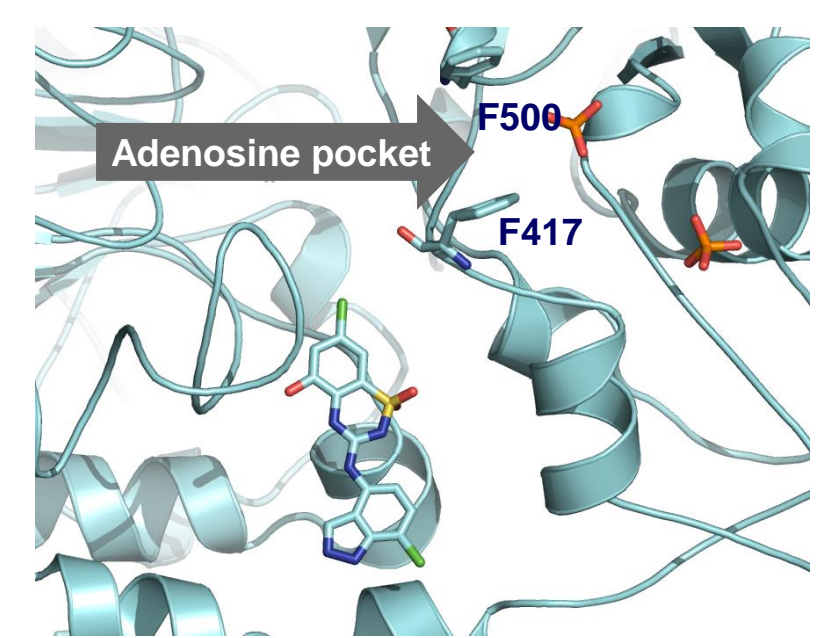
Matrix	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	t <sub>1/2</sub> (h)	AUC (0-inf) (ng.h/mL)	t <sub>1/2</sub> (h)	«Tissue to plasma» AUC <sub>0-inf</sub> ratio
Plasma	81690	0.5	24	174705	2.4 (moderate)	NA
Tumours	12544	0.5	24	56476	2.5 (moderate)	0.32
Brain	1275	0.5	6	2388	1.8 (moderate)	0.013



## Crystallography is providing insights on ligand binding to CD73

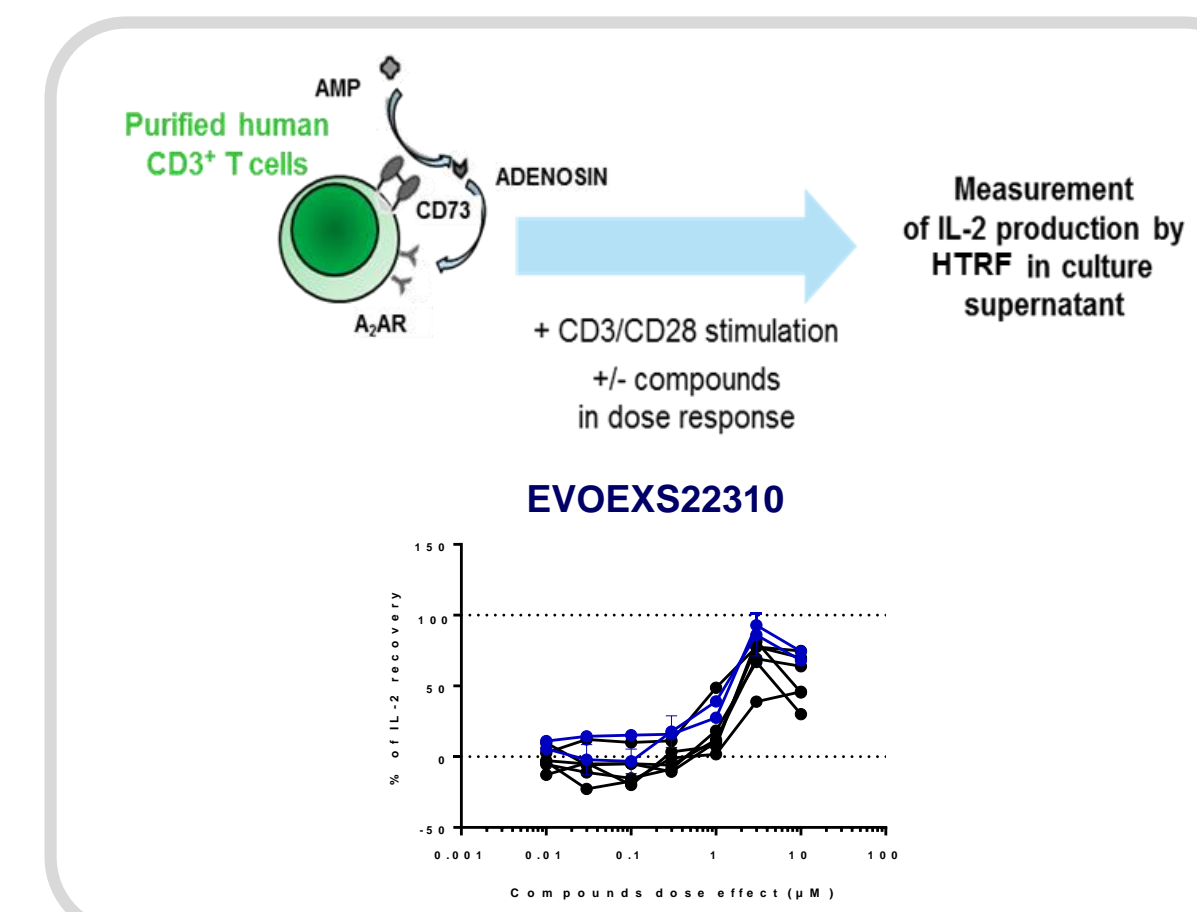
### X-Ray crystal structure of literature compound (from GSK), located away from the adenosine pocket

- X-Ray crystallography is fully enabled for CD73
- The protein can exist in open and closed conformations
- Multiple structures have been obtained for the series under study (resolutions of 1.3-1.9Å)
- Literature inhibitor from GSK (in WO2017098421) binds in a location away from the adenosine pocket
- In contrast EVOEXS compounds bind in the adenosine pocket



## First CD73 inhibitor lead compounds active in the in vitro CD3<sup>+</sup>T-cell functional assay

Human SPR KD (nM)	EVOEXS22343		EVOEXS22310	
	A <sub>2A</sub>	2570	Non Binder	12
CD73	6.3			
Ex vivo activity	EC <sub>50</sub> (nM) (AMP [10 µM])	1127	942	
DMPK	Cl <sub>int,app</sub> H (µL/min/mg)	In progress	15	
	Hep Rat (µL/min/million cells)	In progress	51	
Phys chem	Caco-2 A→B (10 <sup>-6</sup> cm/sec.)	In progress	In progress	
	LogD (pH 7.4)	1.03	0.87	
	Sol pH 1 / 7.4 (µg/ml)	677 / 170	961 / 192	



## Conclusion

### EVOEXS21546 profile

- Adenosinergic Franchise is a new platform in Evotec to accelerate drug discovery in the field of Immuno-oncology
  - Partnership to discover bi-specific small molecule immuno-oncology therapeutics established between Exscientia and Evotec
  - Expansion of existing partnership to discover novel immuno-oncology therapeutics
- Rapid progress has been made on both **specific A<sub>2A</sub>R antagonists** and **CD73 specific molecules** have been identified
- Programme is placed to deliver a **development candidate** by mid-2018
- Potential to also extend bispecific approach within adenosinergic franchise

## Advancement of programs in the adenosinergic franchise

