Abstract #3768

Identification of a novel non-brain penetrant $A_{2A}R$ inhibitor and proof-of-concept of CD73 and $A_{2A}R$/CD73 small-molecule inhibitors for cancer immunotherapy

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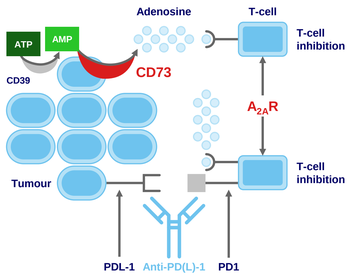
Overview

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

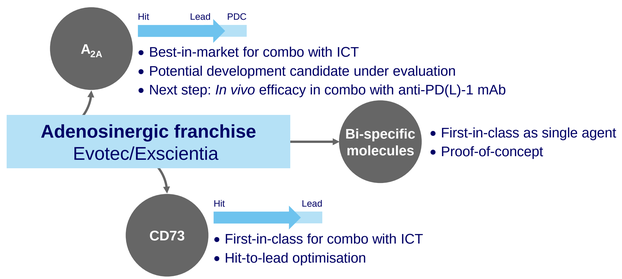
Strategy to develop small molecules inhibiting $A_{2A}R$ and CD73

Effects expected from the $A_{2A}R$ 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

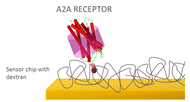


Advancement of programs in the adenosinergic franchise



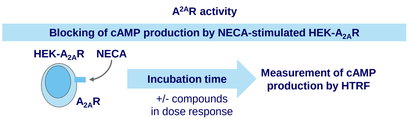
In vitro assays developed at Evotec/Exscientia

SPR screening assay with $A_{2A}R$ bound to the CHIP allows evaluation of fragments or compounds

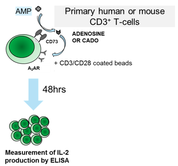


- CD73
  - Optimised assay
- A_{2A}
  - Optimised assay
- A_1
  - Optimised assay
- A_{2B}
  - Optimised assay
- A_3
  - Optimised assay

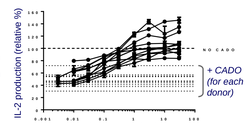
HEK $A_{2A}R$ assay



Ex vivo functional assay on human or mouse $CD3^+$ T cells



Example of $A_{2A}R$ inhibitor on recovery of IL2 production induced by CADO



EVOEXS21546 is a specific, non brain penetrant $A_{2A}R$ antagonist

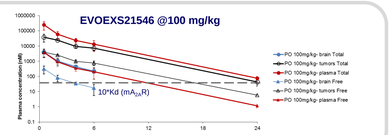
Name	EVOEXS21546
Brain penetrant	NO
Human SPR hA_{2A} KD (nM)	6
Mouse SPR A_{2A} KD (nM)	7
Human SPR A_{2B} A_1/A_3 KD (nM)	1500 / 3130 / 35790
HEK-Human A_{2A} IC_{50} (nM) internal/eurofins	37 / 24
Human A_{2A} functional – EC_{50} (nM)	526
Mouse A_{2A} functional – EC_{50} (nM)	229
Cl_{int}.app (H, µL/min/mg)	10
Caco-2 A->B (10-6 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_{int}.app (µL/min/mg): H/R/M	12 / 25 / 27
Heps Cl_{int}.app (µL/min/10e6 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 5HT2 at 50% (plus A_3: 80% & A_1: 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_{50} > 30 µM
- Ames negative (up to 125 µg/mL)
  - with or without S9 metabolic activation

EVOEXS21546 is a pre-development candidate

Matrix	Cmax (ng/mL)	tmax (h)	tlast (h)	AUC (0-inf) (ng.h/mL)	t1/2 (h)	«Tissue to plasma» AUCo-inf 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



- EVOEXS21546 exhibited a "tumour to plasma" ratio of 0.32 and **"brain to plasma" ratio of 0.013**
- EVOEXS21546 exhibited a **moderate** half-life in plasma (2.4 hrs) and in tumour (2.5 hrs)
- For EVOEXS21546, no side effects were observed after once-daily dosing at 100mg/kg for 12 days

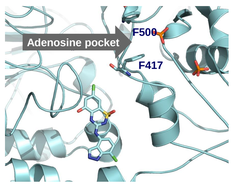
Next steps

- Validate target engagement and dose regimen with PD biomarker strategy
- Evaluate EVOEXS21546 in an in vivo model dependent on the adenosine pathway
- EVOEXS21546 pre-clinical data package to go to INDIGO®, an integrated and rapid process to IND submission, complemented by high-end integrated CMC

Crystallography is providing insights on ligand binding to CD73

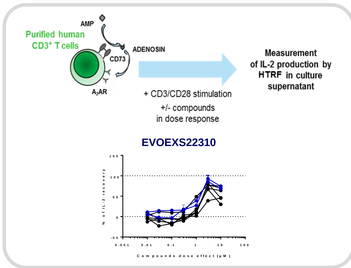
X-Ray crystal structure of literature compound (from GSK), located away from the adenosine pocket

- 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* $CD3^+$T-cell functional assay

		EVOEXS22343	EVOEXS22310
Human SPR KD (nM)	A_{2A}	2570	Non Binder
	CD73	6.3	12
Ex vivo activity	EC_{50} (nM) (AMP [10 µM])	1127	942
DMPK	Clint,app H (µL/min/mg)	In progress	15
	Hep Rat (uL/min/million cells)	In progress	51
Phys chem	Caco-2 A->B (10-6 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 $A_{2A}R$ 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

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