Targeting the adenosine immunosuppressive pathway for cancer immunotherapy with small molecule agents

$A_{2A}R$, CD73 specific and $A_{2A}R$/CD73 Bispecific small molecules for Immuno-Oncology

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Abstract #2634

Overview

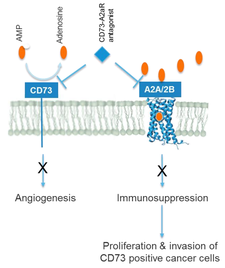
Project concept	Discover specific and bispecific small molecules inhibiting the adenosinergic pathway for immuno-oncology therapies
Strategy	Create patentable high quality assets
Project status	Selection of CD73/$A_{2A}R$ bispecific and $A_{2A}R$ or CD73 specific small molecules
Primary indication	Combination with immune checkpoint therapies for non responder patients
Administration	Oral administration
Biomarker	Patient stratification: CD73 positive tumour Biomarker of activity: Adenosine pathways and CD73 expression

$A_{2A}R$ & CD73 combination prioritized

Modulating adenosine levels in the tumour microenvironment will limit tumour growth and improve anti-tumour immune activity.

Results expected from the $A_{2A}R$/CD73 bispecific molecule

- **Overcoming immunosuppression**
- Enhanced T lymphocyte & NK cell activity
- Decreased tumour cell proliferation
- Inhibiting tumour angiogenesis
- Inducing blood vessel normalization
- Improving blood vessel extravasation



DMTA cycle incorporating Exscientia and Evotec capabilities
Rapid Evaluation: 20 compounds every 2 weeks

Drug design team

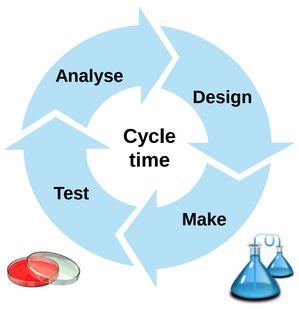
Exscientia evotec

SPR assays

Exscientia

ADME
Phys-chem properties
Biological activities & PK

evotec



Drug design team

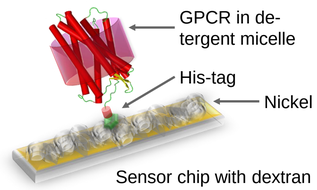
evotec Exscientia

Chemical synthesis (20-30 per cycle)

evotec

GPCRs: World Leading SPR-Driven Fragment Screen

- Both GPCR and Globular Protein Coverage
- Uses wild type GPCR Protein
  - Not thermostabilised
- Identifies orthosteric and allosteric ligands
  - Agonists
  - Antagonists
  - Inverse agonists
- Wide dynamic range KD = mM to pM



Status: full adenosynergic pathway addressed

- A_{2A}
  - Optimised assay
- A_1
  - Optimised assay
- A_{2B}
  - Optimised assay
- A_3
  - Optimised assay
- CD73
  - Optimised assay
- CD39
  - Optimised assay

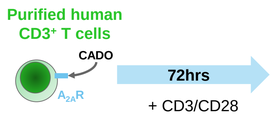
Rapid $A_{2A}R$/CD73 bispecific small molecule discovery
10-criteria selection process

Criteria	Terms	Criteria	Terms
Oral administration	Caco-2 Papp A>B (10-6 cm/sec)	$A_{2A}R$ affinity	6. Kd (SPR assay)
	2. Solubility	$A_{2A}R$ activity	7. cAMP assay HEK-$A_{2A}R$
	3. Log D	CD73 affinity	8. Kd (SPR assay)
Good hepatic clearance	4. Human liver microsomes Clint, app (µL/min/mg)	CD73 activity	9. cAMP competition in CD73 rec.prot
No Cytotoxicity	5. *In vitro* cytotoxicity (HEK)	Adenosine Receptor Selectivity	10. Lowest selectivity SPR assay

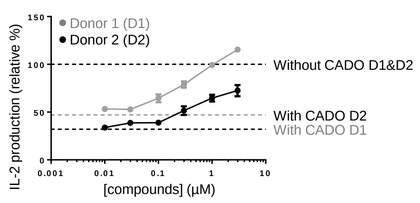
In vitro functional validation

$A_{2A}R$ activity

Recovery of IL-2 production by T cells



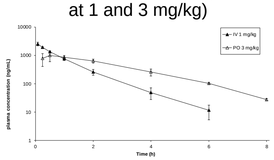
IL2 production induced by 0021546 compound



$A_{2A}R$ specific lead compounds (obtained after 10 months)

Compounds	Preladenant	0021546	0021511
MW	503.6	322.4	321.3
A_{2A} KD (nM)	1	9	1.5
$A_{2B}/A_1/A_3$/CD73 KD (nM)	5180/4390/53280/NA	1500/3130/35790/NA	265/273/6410/NA
HEK A_{2A} AFFINITY (nM)	1.3	38	9.4
A_{2A} PC12 ACTIVITY (nM)	3.1	31	7.8
HEK A_{2A} ACTIVITY (nM)	On going	On going	On going
CD73 KD (nM)	On going	Non Binder	Non binder
Mics Cl_{int}app (µL/min/mg) : H/R/M	41/31/42	14 / 26 / 29	<10
Heps Cl_{int}app (µL/min/10^6 cells) : H/R/M	11/ 20 / 20	4 / 14 / 32	Not tested
PPB % bound : H/R/M (* indicates recovery <55% at 4h)	96.8* /89.3 /97.9*	98.9* /97.7 /83.5*	Not tested
hERG (IC_{50})	> 30 µM	> 30 µM	On going
CYP inhibition (IC_{50}): 2C9/2D6/3A4/1A2/2C19	All > 50 µM	3A4 : 21 µM	Not tested
Caco-2 A->B P_{app} (10^{-6} cm/sec)	11.8	6.4	7.9
Efflux ratio	0.9	1.7	2.1
LogD (pH 7.4)	2.1	1.5	1.3
Sol pH 1 / 7.4 (µg/mL)	>1000 / 2	235 / 12	>1000 / 17

Mean TOTAL plasma concentrations of 0021546
(IV and PO administration to male Sprague Dawley Rat at 1 and 3 mg/kg)



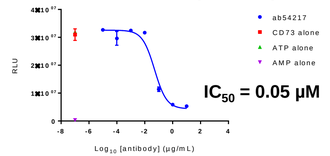
Bioavailability of 42 ± 1%

- Methylcellulose formulation
- Calculated using AUC_{inf}

$A_{2A}R$/CD73 bispecific hits (obtained after 10 months)

Compounds	0000033
MW	330.4
A2A KD (nM)	36
CD73 KD (nM)	1,030
CD73 activity (nM)	36,500
Mics Cl_{int}app (µL/min/mg) : H	39
Caco-2 A->B P_{app} (10^{-6} cm/sec)	7.7
Efflux ratio	0.9
LogD (pH 7.4)	2.8
Sol pH 1 / 7.4 (µg/mL)	20 / 1.4

CD73 recombinant protein assay in competition with cAMP
mAb anti-hCD73 (ab54217)



In vivo proof of concept for lead compounds

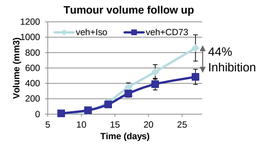
4T1 tumour cell inoculation in BALB/c mice (orthotopic)

Anti-Cd73 mAb injection → Day 28

Group	Treatment
1	Control isotype Ab + vehicle
2	Anti-CD73 mAb

- Efficacy
  - Tumour volume and tumour size
  - Lung metastasis
- Mechanistic read-out
  - TME characterization
  - Immune compartment
- Adenosine pathway quantification
  - Metabolomics

Anti-Cd73 mAb injection



Conclusion & next steps

- Adenosinergic franchise
  - $A_{2A}R$ specific antagonist in Candidate identification phase
  - $A_{2A}R$/CD73 bispecific in Lead identification phase
- Programme well placed to deliver development candidates in 2017
- Potential to deliver CD73 selective inhibitor and to extend bispecific approach to include targets such as CD39
- Platform or specific program open to partnering

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