

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

Pierre Fons¹, Andrew Bell², Stéphanie Versluys¹, Michaël Esquerré¹, Gigliola Mambrini¹, Emilie Pelissier¹, Jérôme Menegotto¹, Anais Duval¹, Florie Bertrand¹, Emilie Pihan¹, Adrian Schreyer², Richard Cox², Joanna Lisztwan¹, Michael Paillasse¹, Iva Navratilova², Françoise Bono¹, Mark Swindells², Mark Whittaker¹, Craig Johnstone¹ and Andrew Hopkins²

¹Evotec, Toulouse, France; ²Exscientia Ltd. Lab 12 Dundee Incubator, James Lindsay Place, Dundee, DD15JJ, United Kingdom



Abstract
#2634

Overview

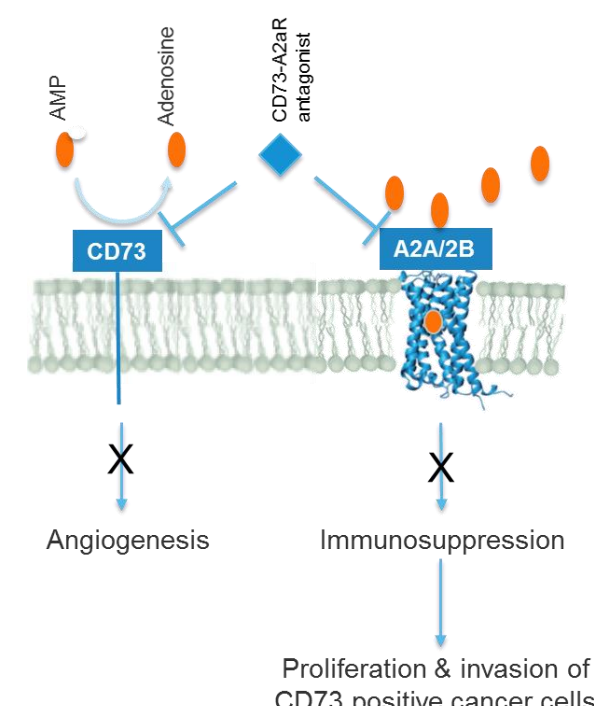
| | |
|---------------------------|---|
| Project concept | Discover specific and bispecific small molecules inhibiting the adenosinergic pathway for immuno-oncology therapies |
| Strategy | Create patentable high quality assets |
| Project status | Selection of CD73/A _{2A} R bispecific and A _{2A} 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

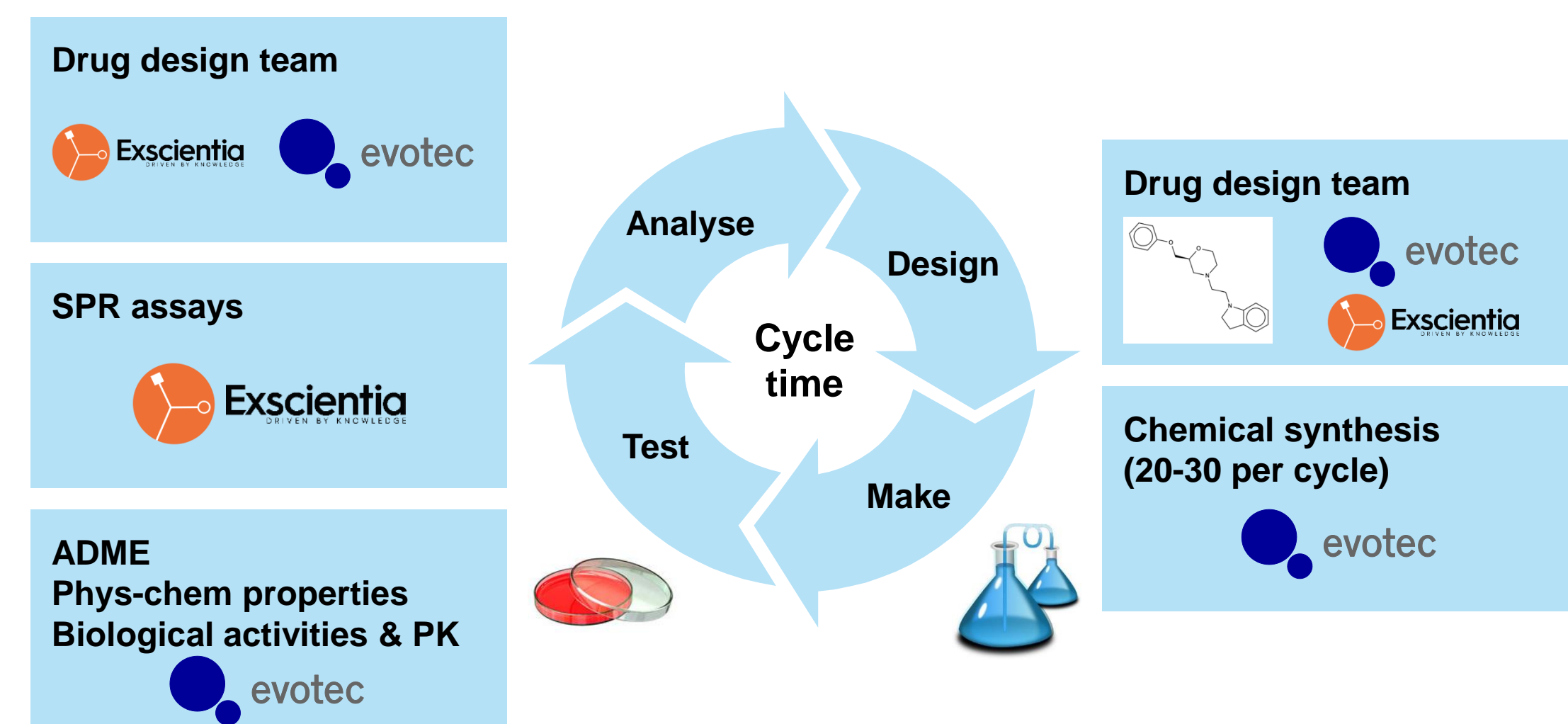
Modulating adenosine levels in the tumour microenvironment will limit tumour growth and improve anti-tumour immune activity.

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

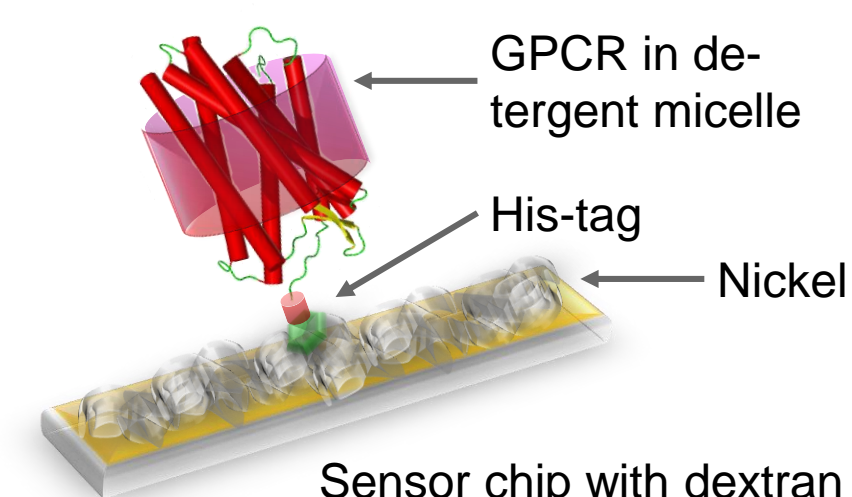


DMTA cycle incorporating Exscientia and Evotec capabilities Rapid Evaluation: 20 compounds every 2 weeks



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 adenosinergic pathway addressed

- A_{2A}
 - Optimised assay
- A₁
 - Optimised assay
- A_{2B}
 - Optimised assay
- A₃
 - Optimised assay
- CD73
 - Optimised assay
- CD39
 - Optimised assay

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

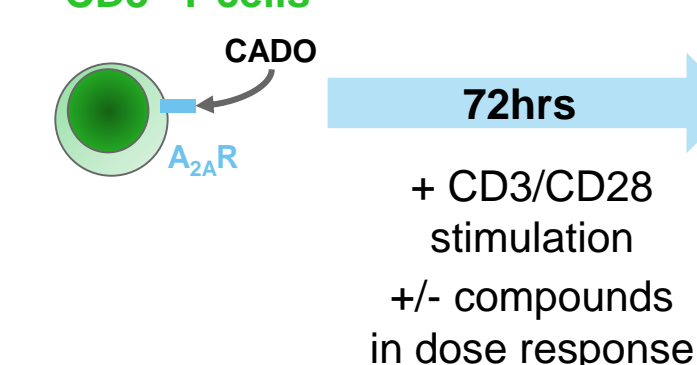
| Criteria | Terms | Criteria | Terms |
|------------------------|--|---------------------------------------|--------------------------------------|
| Oral administration | 1. Caco-2 Papp A>B (10 ⁻⁶ cm/sec) | A _{2A} R affinity | 6. Kd (SPR assay) |
| | 2. Solubility | A _{2A} R activity | 7. cAMP assay 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. <i>In vitro</i> cytotoxicity (HEK) | Adenosine Receptor Selectivity |

In vitro functional validation

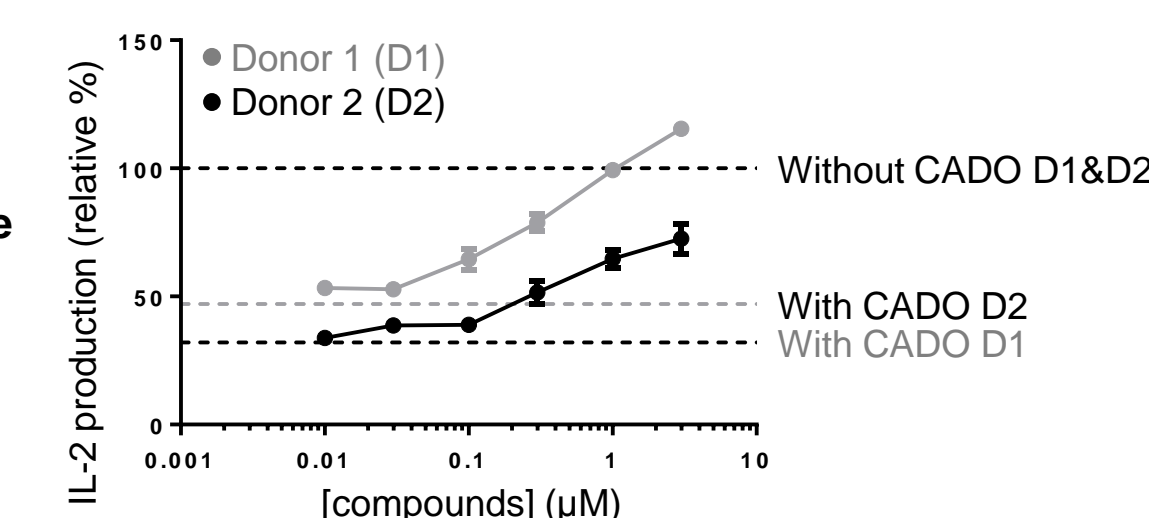
A_{2A}R activity

Recovery of IL-2 production by T cells

Purified human CD3⁺ T cells



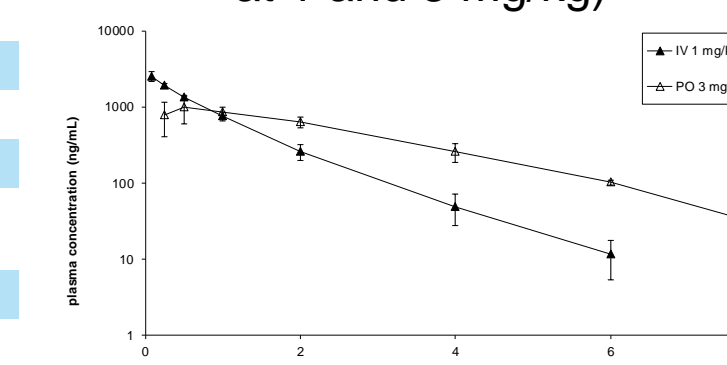
IL2 production induced by 0021546 compound



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)



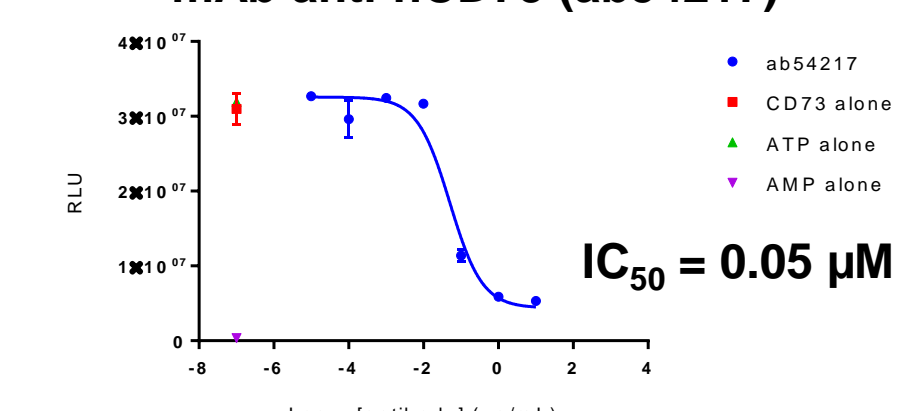
Bioavailability of 42 ± 1%

- Methylcellulose formulation
- Calculated using AUC_{inf}

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

| Compounds | 0000033 |
|--|----------|
| MW | 330.4 |
| A _{2A} 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)

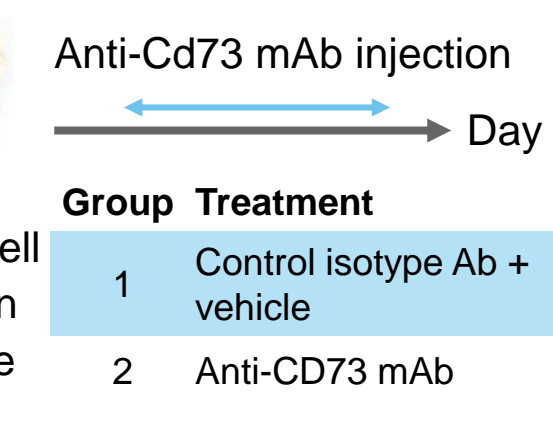


In vivo proof of concept for lead compounds

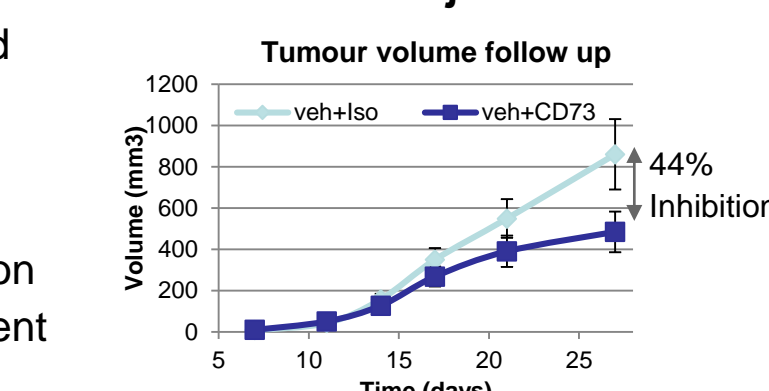
- Efficacy
 - Tumour volume and tumour size
 - Lung metastasis
- Mechanistic read-out
 - TME characterization
 - Immune compartment
- Adenosine pathway quantification
 - Metabolomics



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



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