TRANSLATIONAL PHARMACOLOGY IN ACTION: THE RESEARCH AND DEVELOPMENT OF DARIGABAT, AN α2/3/5 SUBTYPE-SELECTIVE $GABA_A$ RECEPTOR POSITIVE ALLOSTERIC MODULATOR

RACHEL GURRELL

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Translational pharmacology in action: the research and development of darigabat, an α2/3/5-subtype selective $GABA_A$ receptor positive allosteric modulator

Rachel Gurrell

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Supervisors:

Dr Francesca Mackenzie

Dr Joan Jarman

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Acknowledgements

It is a privilege to have this opportunity to re-examine my work in neuroscience drug research and development over 20 years. This journey would never have begun or have been as rewarding without the encouragement and support of many key individuals.

Firstly, I thank my husband and children. I often questioned the wisdom of initiating write up of a PhD thesis during a worldwide pandemic but as a family we truly worked as a team to homeschool, work, and make the mundane special. My children went about their home-schooling with such motivation despite the challenges and made me very proud.

My friend and colleague Dr Richard Butt deserves particular recognition as someone who was and is still influential in my work. I recognise the support given from my colleagues Dr John Renger and Dr Ray Sanchez. They put their trust in me to lead darigabat and encouraged me to start on this journey in the first place.

The supervision and guidance of Dr Francesca Mackenzie and Dr Joan Jarman throughout this process are acknowledged and appreciated.

Lastly, my thanks are due to all those individuals whom I have been privileged to know and work with on this drug program, and those that participated in clinical trials with darigabat. Their contribution to my own growth and to increasing our understanding of $GABA_A$ receptor pharmacology is immeasurable.

Rachel

Abstract

Benzodiazepines (BZDs), non-selective positive allosteric modulators (PAMs) of $GABA_A$ receptors, are highly efficacious across a range of therapeutic indications. However, even at low receptor occupancy (RO), BZDs are associated with significant side effects such as somnolence and are liable to abuse and to the development of tachyphylaxis, all of which limit their clinical utility, particularly in chronic indications such as epilepsy. As many of these undesirable properties are mediated by α1 subunit-containing $GABA_A$ receptors, there has been a concerted effort to develop α2/3/5 subtype selective PAMs for chronic treatment of a range of neurological and neuropsychiatric disorders.

This thesis highlights the biomarker-enabled approach taken in the research and development program of darigabat, a compound identified as demonstrating the desired functional selectivity for $GABA_A$ receptors containing α2/3/5 subunits, with negligible activity at α1 subunits. The wide-ranging biomarker toolkit employed in both preclinical and clinical studies included measures of receptor occupancy, pharmacodynamic activity, and efficacy, and was designed to answer some key research questions. These included demonstrating the translation of the *in vitro* darigabat profile to the clinical profile; identifying attributes that differentiated $GABA_A$ receptor subtype-selective PAMs from BZDs (a critical requirement for the continued development of darigabat); understanding the relationship between darigabat dose, occupancy, and pharmacology; and has provided some understanding of the potential utility of darigabat in a range of CNS disorders.

This work, which spans more than 10 years, has not only identified the therapeutic potential of darigabat, which is currently in Phase 2 clinical trials in focal epilepsy and Phase 1 clinical trials in panic, but has furthered our understanding of $GABA_A$ receptor pharmacology and the promise these compounds hold for the treatment of serious and disabling disorders.

List of Abbreviations

Abbreviation	Definition
AE	Adverse event
AED	Antiepileptic drug
AESI	Adverse event of special interest
AUC	Area under the curve
BID	Twice daily
BZD	Benzodiazepine
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
CI	Confidence interval
CLBP	Chronic low back pain
C_{max}	Maximum concentration
CNS	Central nervous system
CPM	Conditioned pain modulation
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSST	Digit Symbol Substitution Test
EEG	Electroencephalogram
EMG	Electromyogram
ESS	Epworth Sleepiness Scale
GABA	Gamma aminobutyric acid
GAD	Generalised anxiety disorder
GAERS	Genetic absence epilepsy rat from Strasbourg
FDA	Food and Drug Administration
FIH	First-in-human
HAM-A	Hamilton Anxiety
HVLT-R	Hopkins Verbal Learning Test - Revised
ICH	International Conference Harmonisation
iCPM	Inhibitory conditioned pain modulation
IPS	Intermittent photic stimulation
LBPI	Low back pain intensity

Abbreviation	Definition
LSMean	Least Square mean
MAD	Multiple ascending dose
MMRM	Mixed-model repeated measures
MN	Micronucleus
MN^{CD71+}	Micronucleus-containing young reticulocytes
MOA	Mechanism of action
mCIWA-B	Modified version of Clinical Institute Withdrawal Assessment Scale - Benzodiazepines
NAM	Negative allosteric modulator
NMDA	N-methyl-D-aspartic acid
NME	New molecular entity
NOEL	No observed effect level
NRS	Numeric rating scale
NSAID	Non-steroidal anti-inflammatory drug
OLE	Open label extension
PAM	Positive allosteric modulator
PD	Pharmacodynamic
PDT	Pain detection threshold
PET	Positron emission tomography
PK	Pharmacokinetic
POC	Proof-of-concept
POP	Proof-of-principle
PPR	Photoparoxysmal response
PSL-IV	Panic Symptom List-IV
PTT	Pain tolerance threshold
PTZ	Pentylenetetrazol
PWT	Paw withdrawal threshold
qEEG	Quantitative electroencephalogram
RMDQ	Roland Morris Disability Questionnaire
RO	Receptor occupancy
Rratio	Response ratio

Abbreviation	Definition
SAR	Structure-activity relationship
SEM	Standard error of the mean
SPR	Standardised photosensitivity range
SPV	Saccadic peak velocity
SUDEP	Sudden-unexpected death in epilepsy
SWD	Spike-and-wave discharge
TCA	Tricyclic antidepressant
TEAE	Treatment-emergent adverse event
$t_{1/2}$	Apparent terminal half-life
t_{max}	Time to reach maximum concentration
UVB	Ultraviolet radiation B
VAS	Visual analogue scale
VVLT	Visual verbal learning test

1 INTRODUCTION

Rachel Gurrell – expert in drug research and development, specialising in biomarkers, $GABA_A$ receptor pharmacology and Darigabat Scientific Lead

Rachel Gurrell has been working in the pharmaceutical industry for more than 20 years. Her first degree was in Pharmacology and as an undergraduate she secured an industrial placement and a summer scholarship within the pharmaceutical industry which influenced her career path. Following graduation, she accepted a place on a graduate training scheme on Merck, Sharpe and Dohme which was focused on neuroscience. During that time, she began learning the necessary skills to design and execute experiments to assess novel compounds of varying mechanisms of action in early research to treat pain, anxiety, and depression. In 2002 she moved to Pfizer, a tenure that lasted ~ 16 years and one that provided an opportunity to continue to further develop her work in nonclinical models of pain, work in the translational biomarker space utilising models both at the bench and in the clinic, and design clinical trials to understand the therapeutic potential of several different drugs of different mechanisms of actions (MOA). In approximately 2007 she became Exploratory Project Lead of a new program to identify subtype-selective $GABA_A$ receptor positive allosteric modulators (PAMs). Her career path has intersected with the needs of the program until the present day, from designing nonclinical efficacy studies early in her career, designing and executing rodent electroencephalogram (EEG) biomarker studies, designing clinical biomarker and efficacy studies, and later in her career at Pfizer providing project leadership and championing the program. In her current role as Darigabat Scientific Lead at Cerevel Therapeutics she continues to provide project leadership and develop this drug program to ensure that the key scientific questions continue to be answered.

The views expressed in this thesis are solely those of the author and do not necessarily represent those of Cerevel Therapeutics, Pfizer Limited, or any other organisation.

1.1 A Personal Note

Both a love of the pharmacology course at the University of Sheffield and a depleted student grant meant that as an undergraduate, opportunities were sought to learn a trade and earn a wage during an industrial placement year and during summer holidays at pharmaceutical companies. Those positive experiences and meeting individuals with a passion for the science and the research programs they worked on inspired me to follow a career path in the pharmaceutical industry. The final year central nervous system module delivered by Dr Godfrey Collins at the University of Sheffield was the stimulus resulting in a passion for neuroscience, which was further motivated following my father's car accident-induced spinal cord injury which resulted in tetraplegia, him suffering significant pain and his subsequent participation in a clinical trial of a novel analgesic.

Overtime there was realisation that behind various research and development teams made up of dozens of people with differing expertise there is often one individual whose passion for the project drove it forward to ensure that the key scientific questions were answered via quality research. About 6 years ago, I realised that that person was me, contesting for resource to further understand the science which will hopefully be rewarded with a new treatment option for patients.

The portfolio of published work includes:

- 10 journal publications on nonclinical and clinical studies in several neuroscience clinical indications.
- Abstracts from poster presentations at conferences.
- Platform presentations at conferences.

The work will be examined to illustrate the following themes:

- Evolving knowledge of $GABA_A$ receptor pharmacology.
- Use of biomarkers in research and development.
- Safety and efficacy of darigabat, an $\alpha 2/3/5$ subtype-selective $GABA_A$ receptor positive allosteric modulator.

Examples of the published work will also be used:

- To indicate the impact and career context of Rachel Gurrell's work in $GABA_A$ receptor pharmacology.
- To illustrate the utility of biomarkers in answering key research questions.

1.2 Professional experience

1.2.1 Overview

B.Sc. HONS in Pharmacology, University of Sheffield, UK (**1996 – 2000)**

- Including 1-year industrial placement at Rhone-Poulenc Rorer.
- Including 8-week summer placement at Pfizer Ltd.

Merck, Sharp and Dohme, UK (January 2000 – December 2002)

***In vivo* graduate trainee**

- Utilising a range of behavioural nonclinical assay and endpoints in pain, anxiety, and depression to identify and develop targets for drug discovery.

Pfizer Global Research & Development (December 2002 – October 2018)

Scientist – Senior Scientist; Exploratory Project Leader and Pain Behavioural Team Leader (December 2002 – February 2009)

- Led a team of scientists optimising and using a range of nonclinical pain models and endpoints.
- Exploratory Project Lead for Pain Research Unit for several different targets, including for a subtype-selective $GABA_A$ receptor PAM. Identify and develop a screening sequence to discover molecules with a profile suitable to be advanced through the portfolio.
- Pfizer lead on a collaboration between Pharma companies to collate nonclinical pain efficacy data for literature standards.
- 6-month secondment to Rinat (Exploratory Project Lead, San Francisco, September 2007 – March 2008).

Senior Scientist/Principal Scientist; Biomarker Lead and Telemetry Team Leader (March 2009 – March 2012)

Telemetry Team Leader developing physiological biomarker models to aid compound selection, including rodent EEG for darigabat. Biomarker Lead responsible for identifying and developing translational biomarkers for a range of pharmacological targets. Key deliverables and achievements included:

- Scientific and strategic oversight across assigned projects.
- As Telemetry Team Leader, development of multiple telemetry models as translatable physiological biomarkers.
- Data driven contribution to aid selection of compounds which subsequently entered clinical development.

Associate Director/Director, Global Clinical Lead/Program Lead, Lead Clinician, Clinical Research (April 2012 – October 2018)

Global Clinical Lead responsible for attaining global endorsement for project funding for an off-strategy asset. Design of clinical plan for development program to New Drug Application (NDA) filing. Lead Clinician responsible for delivering innovative study design and high-quality data. Key deliverables and achievements included:

- As Lead Clinician, led a range of biomarker, early signal of efficacy and proof-of-concept (POC) Phase 1 and Phase 2 studies for darigabat, and other novel compounds. Through an understanding of each scientific area, each protocol was designed to dissect different attributes of the mechanism of action and deliver decision-making milestones for the organisation.
- Clinical leadership of darigabat epilepsy program that delivered robust efficacy in a Phase 2 clinical trial. Identified changes in the regulatory environment and developed a highly streamlined clinical development plan to NDA that improved the value proposition of the indication. Successfully devised and led an Advisory Board of key opinion leaders and cross-team meetings, led the team through technical and

regulatory risk and commercial assessments to successfully win funding for darigabat during time of financial constraint.

- Finalist of the Pfizer Worldwide Research and Development Achievement Awards for work on the epilepsy program.
- As Program Lead worked with team to out-license darigabat by presenting the data and answering questions on the program from investors which resulted in successful transition of the asset to Cerevel Therapeutics.

Owner/Director - Inflexion Pharma Solutions Ltd (October 2018 – May 2021)

- Providing consulting services to the pharmaceutical industry, building on the 20 years of experience gained working within research and development, from bench to clinic.
- Fulltime consulting role as Scientific Lead of Darigabat at Cerevel Therapeutics from October 2018. Defining the overall nonclinical and development strategy, design and authorship of Phase 2 and open label extension protocols to assess efficacy of in adults with drug-resistant focal epilepsy, and development of strategy to characterise darigabat in anxiety disorder, including design of a Phase 1 experimental medicine study. Continued relationship growth with key opinion leaders and delivering presentations at epilepsy-focused meetings. Preparation of documents and presentation to investors and analysts to support funding of company and transition of Cerevel Therapeutics from a limited company to a public company.

Cerevel Therapeutics (March 2021 – current)

Senior Director, Global Scientific Program Lead; Head Translational Medicine and Biomarkers Cerevel Therapeutics Scientific Lead of Darigabat at Cerevel Therapeutics. Translational Medicine and Biomarkers Lead for several discovery and Phase 1 programs responsible for identification of a translational biomarker strategy for a range of pharmacological targets and clinical indications.

1.2.2 Key Research Outputs

A summary of key publications, conference and poster presentations associated with the darigabat (formerly known as CVL-865 and PF-06372865) research and development

program is in **Table 1-1**. **Figure 1-1** illustrates the darigabat project roles and associated publications. The publications associated with this PhD by publication are referenced in **Appendix 1**.

Throughout this work references in which the thesis author is a listed as an author on the publications are cited in blue.

Table 1-1 Summary of key research outputs of Rachel Gurrell

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
Nickolls, S., Mace, H., Fish, R., Edye, M., **Gurrell, R**., Ivarsson, M., Pitcher, T., Tanimoto-Mori, S., Richardson, D., Sweatman, C., Nicholson, J., Ward, C., Jinks, J., Bell, C., Young, K., Rees, H., Moss, A., Kinloch, R. and McMurray, G. (2011) 'A comparison of the α2/3/5 selective positive allosteric modulators L-838,417 and TPA023 in nonclinical models of inflammatory and neuropathic pain', Advances in Pharmacological Sciences, 2011, p. 608912. doi:10.1155/2011/608912.	Publication 2011. 31 citations.	Characterisation of the nonclinical efficacy of subtype selective PAM tool compounds in inflammatory and neuropathic pain models.	Design, execution, analysis, and interpretation of rodent EEG studies. Contribution to manuscript development.	None included, but confirmation provided to PhD supervisors by Dr Magnus Ivarsson.
Okkerse, P., van Amerongen, G., de Kam, M.L., Stevens, J., Butt, R.P., **Gurrell, R**., Dahan, A., van Gerven, J.M., Hay, J.L. and Groeneveld, G.J. (2017) 'The use of a battery of pain models to detect analgesic properties of compounds: a two-part four-way crossover study', British Journal of Clinical Pharmacology, 83(5), pp. 976-990. doi:10.1111/bcp.13183.	Publication 2017. 24 citations.	This methodology paper describes the development of a healthy participant clinical trial designed to understand the analgesic potential of drugs early in development using a range of clinically used analgesics.	Contribution to concept, design, and interpretation of PainCart methodology clinical trial.	**RG** and colleagues reviewed and commented on the manuscript. **RG** and colleagues designed the research.

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
			Reviewed and commented on the manuscript.	
Nickolls, S.A., **Gurrell, R**., van Amerongen, G., Kammonen, J., Cao, L., Brown, A.R., Stead, C., Mead, A., Watson, C., Hsu, C., Owen, R.M., Pike, A., Fish, R.L., Chen, L., Qiu, R., Morris, E.D., Feng, G., Whitlock, M., Gorman, D., van Gerven, J., Reynolds, D.S., Dua, P. and Butt, R.P. (2018) 'Pharmacology in translation: the nonclinical and early clinical profile of the novel α2/3 functionally selective $GABA_A$ receptor positive allosteric modulator PF-06372865', British Journal of Pharmacology, 175(4), pp. 708-725. doi:10.1111/bph.14119.	Publication 2018. 28 citations.	This publication includes data from a wide range of *in vitro* and *in vivo* studies with darigabat, and clinical pharmacodynamic data. It demonstrates how the MOA contributes to the pharmacology profile and how it is differentiated from a BZD using rational selection of assays/models/endpoints from the bench to the clinic.	Design, execution, and interpretation of rodent EEG study. Contributed to design and interpretation of the first-in-human and PET clinical trials. Co-first authorship of manuscript.	D.S.R., P.D. and R.P.B. designed the research studies. **R.G**., J.K., L.C., A.R.B., A.M., C.H., S.A.N., C.W., R.M.O., D.S.R., R.L.F., A.P., R.Q., M.W., D.G. and P.D. analysed the data. S.A.N., **R.G**., D.S.R. and P.D. wrote the paper.

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
Gurrell, R., Dua, P., Feng, G., Sudworth, M., Whitlock, M., Reynolds, D.S. and Butt, R.P. (2018) 'A randomised, placebo-controlled clinical trial with the α2/3/5 subunit selective $GABA_A$ positive allosteric modulator PF-06372865 in patients with chronic low back pain', Pain, 159(9), pp. 1742-1751. doi:10.1097/j.pain.0000000000001267.	Publication 2018. 15 citations.	A clinical trial in patients with chronic low back pain showed no efficacy of darigabat up to a dose which achieved approximately 50% RO. The publication explores the potential reasons for the lack of efficacy observed.	Contribution to design, lead the execution of the clinical trial and interpretation of data. First author of the manuscript.	No statement included, but confirmation provided to PhD supervisors by Dr Richard Butt.
van Amerongen, G., Siebenga, P.S., **Gurrell, R**., Dua, P., Whitlock, M., Gorman, D., Okkerse, P., Hay, J.L., Butt, R.P. and Groeneveld, G.J. (2019) 'Analgesic potential of PF-06372865, an α2/α3/α5 subtype-selective $GABA_A$ partial agonist, in humans', British Journal of Anaesthesia, 123(2), pp. e194-e203. doi:10.1016/j.bja.2018.12.006.	Publication 2019. 9 citations.	Description of data generated in a battery of pain-related tasks in healthy participants which was designed to elucidate the analgesic potential of darigabat.	Contribution to concept, design, and interpretation of PainCart darigabat efficacy clinical trial. Contribution to development of manuscript.	**Drafting of the manuscript: all authors.** Study concept and design: GvA, JH, GJG, **RG**, PO, RB, PD. Acquisition of data: GvA, JH, GJG, PS.

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
				Data analysis: PD, MW, DG.
Duveau, V., Buhl, D.L., Evrard, A., Ruggiero, C., Mande-Niedergang, B., Roucard, C. and **Gurrell, R**. (2019) 'Pronounced antiepileptic activity of the subtype-selective $GABA_A$-positive allosteric modulator PF-06372865 in the GAERS absence epilepsy model', CNS Neuroscience & Therapeutics, 25(2), pp. 255-260. doi:10.1111/cns.13046.	Publication 2019. 13 citations.	Demonstration of efficacy of darigabat in a nonclinical model of generalized epilepsy. The totality of the data in nonclinical epilepsy models shows that darigabat has a broad spectrum of efficacy.	Concept, design, and interpretation of study. Co-authoring of manuscript.	Author contribution was submitted to the journal but not published.
Owen, R.M., Blakemore, D., Cao, L., Flanagan, N., Fish, R., Gibson, K.R., **Gurrell, R**., Huh, C.W., Kammonen, J., Mortimer-Cassen, E., Nickolls, S.A., Omoto, K., Owen, D., Pike, A., Pryde, D.C., Reynolds, D.S., Roeloffs, R., Rose, C., Stead, C., Takeuchi, M., Warmus, J.S. and Watson, C. (2019) 'Design and identification of a novel, functionally subtype selective $GABA_A$ positive allosteric modulator (PF-06372865)', Journal of Medicinal Chemistry, 62(12),	Publication 2019. 10 citations.	Design and identification of darigabat, including description of the physiochemical properties and efficacy in nonclinical animal models of pain, anxiety, and epilepsy.	Design and interpretation of nonclinical pain and epilepsy studies. Contribution to manuscript development.	The manuscript was written through contributions of all authors.

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
pp. 5773-5796. doi:10.1021/acs.jmedchem.9b00322.				
Gurrell, R., Gorman, D., Whitlock, M., Ogden, A., Reynolds, D.S., DiVentura, B., Abou-Khalil, B., Gelfand, M., Pollard, J., Hogan, R.E., Krauss, G., Sperling, M., Vazquez, B., Wechsler, R.T., Friedman, D., Butt, R.P. and French, J. (2019) 'Photosensitive epilepsy: robust clinical efficacy of a selective GABA potentiator', Neurology, 92(15), pp. e1786-e1795. doi:10.1212/WNL.0000000000007271.	Publication 2019. 12 citations.	First demonstration of anticonvulsant potential of a subtype selective $GABA_A$ receptor PAM was observed in patients with photosensitive epilepsy.	Concept, design, and interpretation of clinical efficacy study. Lead authorship of manuscript.	**RG:** Design and conceptualised study; interpreted the data; drafted the manuscript for intellectual content.
Bialer, M., Johannessen, S.I., Koepp, M.J., Levy, R.H., Perucca, E., Perucca, P., Tomson, T. and White, H.S. (2020) 'Progress report on new antiepileptic drugs: a summary of the Fifteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XV). I. Drugs in nonclinical and early clinical development', Epilepsia, 61(11), pp. 2340-2364. doi:10.1111/epi.16725. **Section on darigabat; Gurrell R.**	Publication 2020. 10 citations.	Summary of selected data to date, including pharmacology, nonclinical and clinical efficacy in epilepsy, pharmacokinetics, and RO.	Sole authorship of darigabat section of manuscript.	Sole author of darigabat section of manuscript.

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
Gurrell, R., Whitlock, M., Wei, H., Shen, Z. and Ogden, A. (2021) 'Safety, tolerability, and pharmacokinetics of multiple repeated oral doses of the α2/3/5-subtype selective $GABA_A$-positive allosteric modulator PF-06372865 in healthy volunteers', Clinical Pharmacology in Drug Development. doi:10.1002/cpdd.912.	Publication 2021. 0 citations.	Describes safety and tolerability data observed with high RO darigabat in a MAD study in healthy participants.	Concept, design, and interpretation of clinical multiple ascending dose study. Lead authorship of manuscript.	**RG,** MW, HW, ZS and AO contributing to the design, analysis and interpretation of data associated with this clinical trial and contributed to the development of this manuscript.
Gurrell, R. (February 2018) 'PF-06372865, a novel α2/3/5-subtype selective $GABA_A$ modulator for epilepsy'. Platform Presentation: Epilepsy Pipeline Meeting, San Francisco, USA.	Conference presentation 2018.	Pharmacology and translational data package for darigabat	Presenter	N/A
Gurrell, R. (May 2019) 'PF-06372865, a novel α2/3/5-subtype selective $GABA_A$ modulator for epilepsy'. Platform Presentation: Antiepileptic Drugs and Devices Meeting, Miami, USA.	Conference presentation 2019.	Summary of darigabat research and development program	Presenter	N/A

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
Gurrell, R. (July 2020) 'CVL-865'. Platform Presentation: Eilat XV Virtual Conference.	Conference presentation 2020.	Summary of darigabat pharmacology	Presenter	N/A
Gurrell, R. (August 2020) 'CVL-865'. Platform Presentation: Epilepsy Pipeline virtual Conference.	Conference presentation 2020.	Summary of darigabat efficacy and safety profile and planned clinical trials.	Presenter	N/A
Gurrell, R., Gorman, D., Whitlock, M., Ogden, A., Reynolds, D.S., DiVentura, B., Abou-Khalil, B., Gelfand, M., Pollard, J., Hogan, R.E., Krauss, G., Sperling, M., Vazquez, B., Wechsler, R.T., Friedman, D., Butt, R.P. and French, J. (December 2017) 'PF-06372865, an α2/3/5-subtype selective $GABA_A$ partial positive allosteric modulator has promising efficacy in the photosensitivity model'. Poster Presentation: American Epilepsy Society Conference, Washington DC, USA.	Poster presentation 2017.	Photoepilepsy model	Presenter	N/A
Buhl, D.L., DaSilva, J.K., Tyszkiewicz, C., Goody, S.M.G., Mead, A.M., Morse, D., Weber, M.L., **Gurrell, R**. (December 2017) 'PF-	Poster presentation 2017.	Efficacy of darigabat in nonclinical epilepsy models	Presenter	N/A

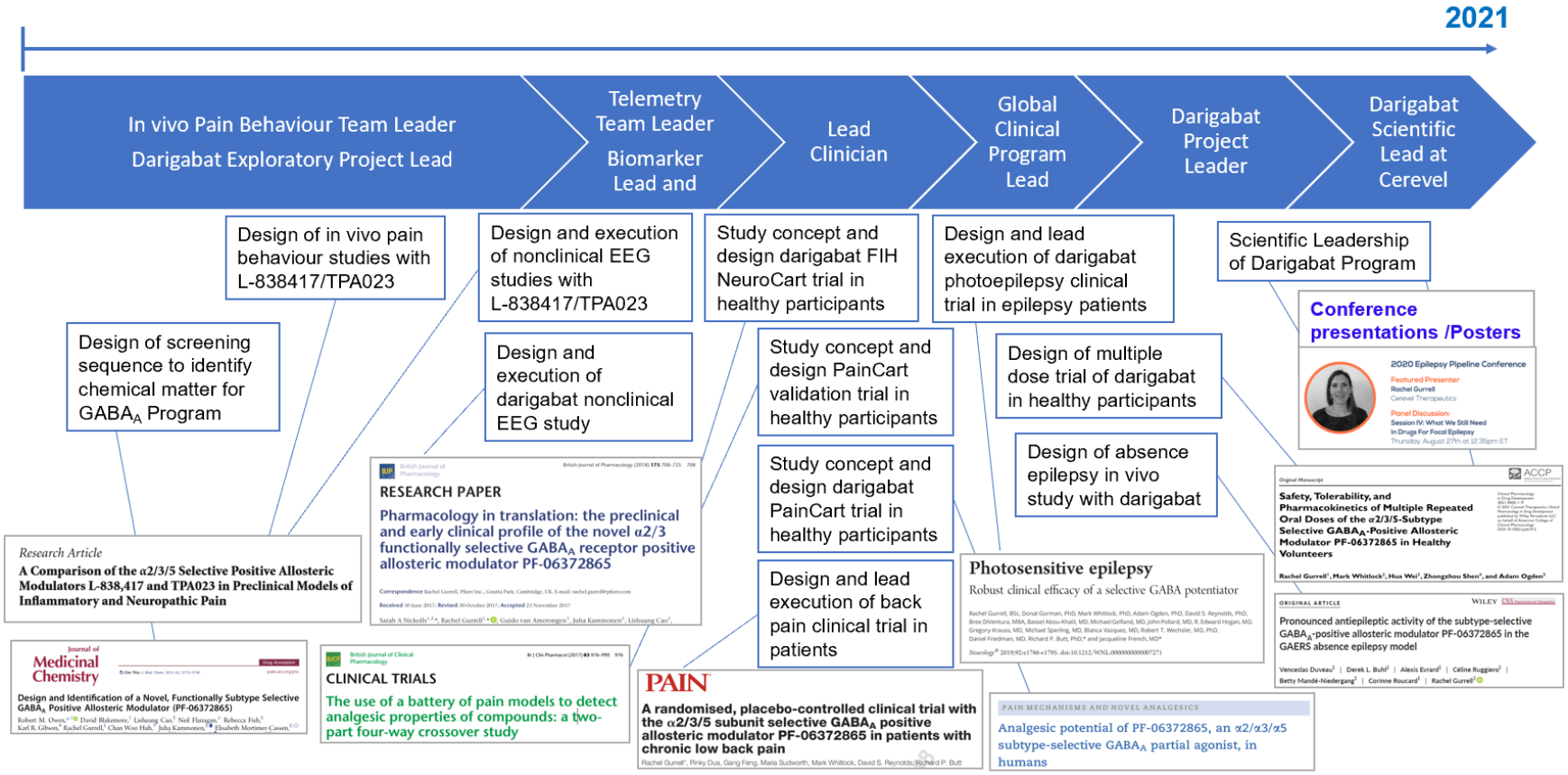
Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
06372865, an α2/3/5-subtype selective $GABA_A$ partial positive allosteric is a potent anticonvulsant in animal models of epilepsy.' Poster Presentation: American Epilepsy Society Conference, Washington DC, USA.				
Gurrell, R., Gorman, D., Whitlock, M., Ogden, A., Reynolds, D.S., DiVentura, B., Abou-Khalil, B., Gelfand, M., Pollard, J., Hogan, R.E., Krauss, G., Sperling, M., Vazquez, B., Wechsler, R.T., Friedman, D., Butt, R.P. and French, J. (September 2019). 'Utility of the double-blind crossover design protocol for proof-of-principle photosensitivity model assessment of PF-06372865.' Poster Presentation: International Society for CNS Clinical trial and Methodology Conference, Copenhagen, Denmark.	Poster presentation 2019.	Photoepilepsy model and study design	Presenter	N/A
Gurrell, R., Whitlock, M., Ogden, A. (December 2019). 'Safety, tolerability, and pharmacokinetics of multiple repeated oral doses of the subtype selective $GABA_A$ positive allosteric modulator PF-06372865 in healthy volunteers.' Poster	Poster presentation 2019.	Safety and tolerability of darigabat in a MAD trial in healthy participants.	Presenter	N/A

Reference	Research output year and number of citations	Summary	Contribution R. Gurrell	Contribution statement in publication
Presentation: American Epilepsy Society Conference, Baltimore, USA.				

Abbreviations: BZD = benzodiazepine; EEG = electroencephalogram; MAD = multiple ascending dose; MOA = mechanism of action; N/A = not applicable; PAM = positive allosteric modulator; PET = positron emission tomography; RO = receptor occupancy.

Select research outputs in chronological order. Publications are listed followed by conference presentations and then poster presentations. Citation numbers are based on google.scholar.com and downloaded on 21st May 2021.

Figure 1-1 Schema demonstrating darigabat project roles and associated publications



Abbreviations: EEG = electroencephalogram.

Schematic highlighting author's roles in the darigabat program and associated publications over time. Timeline is approximate.

1.3 $GABA_A$ Receptors, benzodiazepines, and subunit specificity

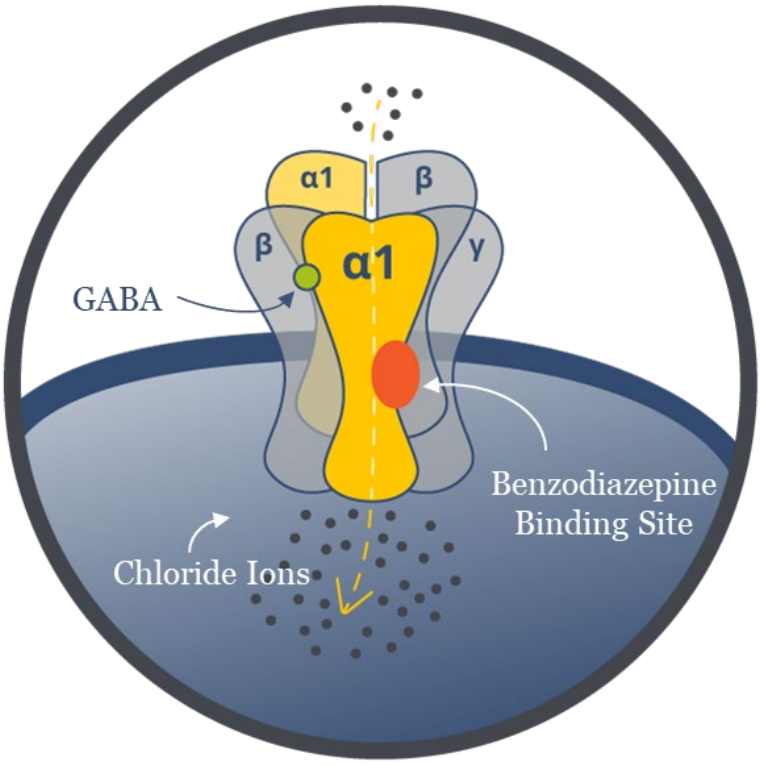
Neuronal signaling via the γ-aminobutyric acid (GABA) type A ($GABA_A$) receptor plays a critical role in a wide range of processes within the central nervous system (CNS). $GABA_A$ receptors are heteropentamers assembled from the 19 members (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3) of the $GABA_A$ receptor family, with the most abundant subtypes comprising α, β, and γ subunits in a 2:2:1 stoichiometry (McKernan and Whiting, 1996).

Approximately 50% of $GABA_A$ receptors in the brain contain α1 subunits and they are widely distributed throughout (McKernan and Whiting, 1996; Sequeira et al., 2019; Maramai et al., 2020). The α2- and α3-containing receptors constitute approximately 35% of total $GABA_A$ receptors and are located throughout the cerebral cortex, with enriched expression of α2 also in the amygdala and spinal cord motoneurons. α5-containing subunits are less abundant, accounting for approximately 4% of total $GABA_A$ receptors and are preferentially expressed in the hippocampus The patterns of expression of $GABA_A$ receptors subunits in the brain is highly stereotypical across healthy controls (Sequeira et al., 2019).

Receptor activation results in an increased membrane chloride conductance, which typically causes an influx of chloride ions and results in membrane hyperpolarisation which dampens down excitability (**Figure 1-2**). Several different classes of pharmacological agents exert their actions on the $GABA_A$ receptor by binding to recognition sites that are distinct from the binding site of the endogenous ligand GABA, including barbiturates, alcohol, BZDs, and neurosteroids (Maramai et al., 2020). Compounds that bind to the BZD binding site are allosteric ligands, that is, they exhibit no intrinsic activity of their own, but potentiate (positive allosteric modulator; PAM) or inhibit (negative allosteric modulator; NAM) the effects of GABA at receptors that contain an α1, α2, α3, or α5 subunit in combination with a γ2 subunit (Dawson et al., 2006).

All the overt effects of BZDs are mediated by $GABA_A$ receptors: sedative, anxiolytic, anticonvulsant, muscle relaxant, addictive, and amnestic. These broad pharmacological effects result in wide ranging clinical utility of BZDs, including induction of presurgical sedation, myorelaxation, and in the treatment of anxiety, pain, and seizures. However,

Figure 1-2 Schematic representation of the arrangement of subunits and GABA and benzodiazepine binding sites in the $GABA_A$ receptor



Schematic representation of the arrangement of subunits in the $GABA_A$ receptor. The $GABA_A$ receptor is a heteropentameric arrangement, with the most common arrangement of subunits consisting of two α, two β, and one γ. The GABA binding site occurs at the interface of the α and β subunits. When the γ subunit is γ2 and the α subunit is either α1, α2, α3, or α5 (but not α4 or α6), a benzodiazepine recognition site is formed at the interface of these subunits. When activated, $GABA_A$ receptors generally permit the flow of chloride ions from outside to inside the neuron, thereby resulting in a hyperpolarisation of the membrane potential that reduces excitability and the probability of the neuron firing further action potentials.

this diverse pharmacology is also associated with significant side effects that limit their clinical utility in some of these populations, even at low RO. Moreover, protracted BZD use can be associated with the development of physical dependence and a tolerance

toward certain aspects of their pharmacology which results, for example, in them being generally unsuitable for prophylactic use in epilepsy patients. Therefore, strategies to develop compounds with BZD-like anxiolytic activity but free from negative attributes such as sedation were sought. The initial wave of these compounds was non-selective and exhibited activity at α1, α2, α3, and, α5-containing $GABA_A$ receptors but displayed lower functional efficacy than classic BZDs, with 'partial' rather than 'full agonist' efficacies. Compounds exhibiting this profile such as bretazenil showed a clear separation between doses demonstrating anxiolysis and those that were sedating in preclinical species, although little dissociation between anxiolysis and sedation was observed in the clinic (Atack, 2003). Furthermore, at that time compounds assumed to be non-selective low functional efficacy $GABA_A$ receptor PAMs such as abercarnil and pagoclone were moved into the clinic despite unclear in vitro functional efficacy profiles and subsequently failed to demonstrate efficacy in anxiety populations. A new approach to develop compounds that selectively targeted specific α subunits was only enabled with the advent of new molecular cloning techniques. These sophisticated molecular studies in which $GABA_A$ receptors containing specific α subunits have been rendered unresponsive to diazepam have elucidated the contribution of those subunits to different aspects of the *in vivo* pharmacology (Rudolph et al., 1999).

These studies, together with subtype-selective tool compounds, have attributed the sedative effects of BZDs to α1 activity (McKernan et al., 2000), anticonvulsant activity to α1/2 subunits (Fradley et al., 2007), anxiolytic and myorelaxant effects to α2/3 (Crestani et al., 2001; Dias et al., 2005; Atack et al., 2006), analgesic effects to α2/3/5 (Knabl et al., 2008), some of the effects on memory function to α1/5 receptors (Roehrs et al., 1994; Dawson et al., 2006), addiction to α1/2 subtypes (Tan et al., 2011; Stephens et al., 2017), and development of efficacy tolerance in epilepsy populations to full agonist activity at the α1 subunit (Vinkers and Olivier, 2012; Cheng et al., 2018) (**Table 1-2**).

Table 1-2 Summary of the $GABA_A$ receptor subtype associated with aspects of benzodiazepine pharmacology

GABA subtype predicted effects:	α1	α2	α3	α5
Anti-convulsant	✓✓	✓✓		
Anxiolysis		✓✓	✓✓	
Analgesia		✓✓	✓	✓✓
Muscle Relaxation		✓✓	✓✓	
Sedation	✓✓			
Cognitive Impairment	✓✓	?	?	✓
Addiction	✓✓	✓		
Abbreviations: ? = unknown; ✓✓ indicates stronger evidence than ✓.				

Although demarcation of the subtypes into BZD pharmacology is without doubt an over-simplification, it however does underpin the hypothesis that compounds with minimal pharmacological activity at α1-containing subtypes, that exhibit potentiation of GABA via α2/3-subtypes, should be non-sedating anxiolytics. The hypothesised role of α1-containing $GABA_A$ receptors is somewhat clinically validated by zolpidem, the α1-subtype preferring sedative hypnotic. As such, there has been a concerted effort to identify α2/3 subtype-selective PAMs, with negligible potentiation of GABA via the α1 subunit, in a bid to treat seizures, pain, and anxiety with an improved side effect profile versus a BZD.

The effort to identify compounds that exhibited the desired profile of selectively binding to the α2- or α3-subunit containing receptors over those containing α1 subunits proved challenging. This is presumably due to the conservation of key binding residue in the BZD

binding site which is located at the α/γ interface of the $GABA_A$ receptor complex (Morlock and Czajkowski, 2011; Ain et al., 2016; **Figure 1-2**). Instead, it was observed that selectivity could be more readily obtained functionally, i.e., a given compound binds to a different receptor subunit combination with comparable affinity but promotes very different levels of functional activity (**Table 1-3**). Ultimately this approach proved successful and has led to the identification of a broad range of both clinical and nonclinical compounds (*vide infra*). However, to date, no new approved drugs have resulted from this work.

Whilst there is robust nonclinical evidence that negligible α1-potentiation is the desired *in vitro* profile of subtype-selective $GABA_A$ receptor PAMs, translation of minimal α1-potentiation *in vitro* to a lack of dose-limiting sedation or somnolence in the clinic has not always proved to be the case. For example, the $GABA_A$ receptor PAM MK-0343 is reported to exhibit α3 > α2 = α5 > α1 subtype-selectivity (**Table 1-3**), with low intrinsic efficacy of the latter (~20% of diazepam) and was demonstrated to be anxiolytic *in vivo* at doses corresponding to ~35–65% receptor occupancy (RO) (Atack, 2010). However, dose-limiting sedation was observed in the clinic at levels of RO below the limit of detection (de Haas et al., 2008). Subsequent compounds which exhibited lower functional efficacy at α1 subunits than MK-0343, TPA023, and TPA023B, also possessed dose-limiting adverse events (AEs) including drowsiness which precluded dosing beyond approximately 60% RO (Atack, 2010). Furthermore, ocinaplon (subsequently discontinued) was found to be a non-sedating anxiolytic in two trials in patients with generalised anxiety disorder (GAD), which was at odds with the *in vitro* profile which showed significant functional efficacy at α1 (Lippa et al., 2005; Berezhnoy et al. 2008). The reasons for the differences in the clinical tolerability are not clear and there remains an incomplete understanding of the effects of PAMs on $GABA_A$ receptors at the biophysical level. However, these tool compounds have been valuable to furthering our understanding of $GABA_A$ receptor pharmacology and of utility to “validate” in-house assays and make comparisons with newly identified molecules from medicinal chemistry screening efforts. As such, in approximately 2006, Pfizer embarked on an exploratory program with the objective of identifying α2/3-selective compounds with minimal α1-

Table 1-3 Summary of the representative *in vitro* properties of select $GABA_A$ receptor PAMs

Compound	Binding selectivity	Functional selectivity
Diazepam	α1=α2=α3=α5	α1=α2=α3=α5
Darigabat	**α1>α2=α3>α5**	**α2>α3=α5>α1**
Bretazenil	α1=α2=α3=α5	α1=α2=α3=α5
Ocinaplon	α1=α2=α3=α5	α1>α2=α3=α5
L-838417	α1=α2=α3>α5	α2=α3=α5>α1
MK-0343	α1=α2=α3=α5	α3>α2=α5>α1
TPA023	α1=α2=α3=α5	α3>α2>α5>α1
TPA023B	α1=α2=α3=α5	α3>α2=α5>α1
AZD7325	α1=α2>α3>α5	α2=α3>α5>α1
AZD6280	α1>α2>α3>α5	α2=α3>α5>α1
NS11821	α1=α5>α3>α2	α3=α5>α2>α1
NS11394	α5=α1>α2>α3	α5>α3>α2>α1

Abbreviation: PAM = positive allosteric modulator.

In vitro binding and functional selectivity profiles for select clinical and nonclinical $GABA_A$ receptor PAMs relative to different α subunit subtypes. As experimental methodologies differ between laboratories this is intended as a guide to approximate compound profiles. Within approximately 2-fold in vitro binding affinity or functional efficacy was consider approximately equivalent. (Atack, 2003; Atack, 2010; Nickolls et al., 2018; Maramai et al., 2020). More information on the *in vitro* profile of darigabat is in **Table 1-4**.

activity and designed a research and development program to identify differentiation from both BZDs, and other subtype-selective PAMs that went before it.

1.3.1 *In vitro* profile of darigabat, an α2/3/5-selective $GABA_A$ receptor positive allosteric modulator

The target *in vitro* profile for potential candidates from the Pfizer medicinal chemistry screening work was derived from an understanding of other subtype-selective PAMs that had entered clinical development. MK-0343, which exhibits selectivity for α2 over α1 in functional assays but still shows moderate α1-activity was markedly sedative in the clinic (Atack, 2010). In contrast, TPA023 and TPA023B which both show selectivity as well as decreased efficacy at α1 relative to MK-0343, showed reduced sedative effects in clinical trials (de Haas et al., 2007; Atack, 2009; Atack et al., 2011). Furthermore, TPA023 also showed preliminary evidence of efficacy in both anxiety and schizophrenia patients (Atack, 2009). Based on this analysis, a maximal α1 enhancement of the GABA response in the *in vitro* system was set at approximately 20% and a minimum α2 enhancement of 50% as the target PAM profile for any potential candidates (Owen et al., 2019).

Darigabat (formerly known as PF-06372865 and CVL-865) was identified as described in detail in Owen et al., 2019, using the *in vitro* screening assays described in Nickolls et al., 2018. It is a novel small molecule high affinity ligand for the BZD site of the $GABA_A$ receptor, with functional, but not binding selectivity for receptors containing α2/3/5 subunits compared with those containing an α1 subunit (Nickolls et al, 2018).

The affinity of darigabat for the BZD site of $GABA_A$ receptors was determined in competition-binding experiments, versus [3H]-flumazenil (receptors containing α1/2/3/5 subunits) or [3H]Ro15-4513 (receptors containing α4/6 subunits), in membranes from recombinant cell lines expressing $GABA_A$ receptors containing specific α subunits (Nickolls et al., 2018; **Table 1-4**). Darigabat was determined to be a high-affinity ligand at $GABA_A$ receptors containing an α1, α2, α3, or α5 subunit but had no affinity for $GABA_A$ receptors containing an α4 or α6 subunit, which is typical of BZD site ligands as α4/6-containing $GABA_A$ receptors do not have a BZD-binding site. Darigabat did not display identical affinity at α1/2/3/5 containing $GABA_A$ receptors and had a rank order of affinity α1 > α3 ≈ α2 > α5 (Nickolls et al., 2018).

Table 1-4 *In vitro* properties of darigabat

Subtype	Affinity (nM (CIs))	E_{max} manual patch (% potentiation of GABA EC_{10})
Human α1β3γ2	0.18 (0.09-0.35)	20.2 ± 3.4
Human α2β2γ2	2.92 (1.03-8.24)	123.7 ± 12.3
Human α3β3γ2	1.06 (0.56-2.00)	144.6 ± 20.4
Human α5β2γ2	18.04 (7.28-44.71)	95.0 ± 11.5
Human α4β3γ2	>19000	n.d.
Human α6β3γ2	>19000	n.d.

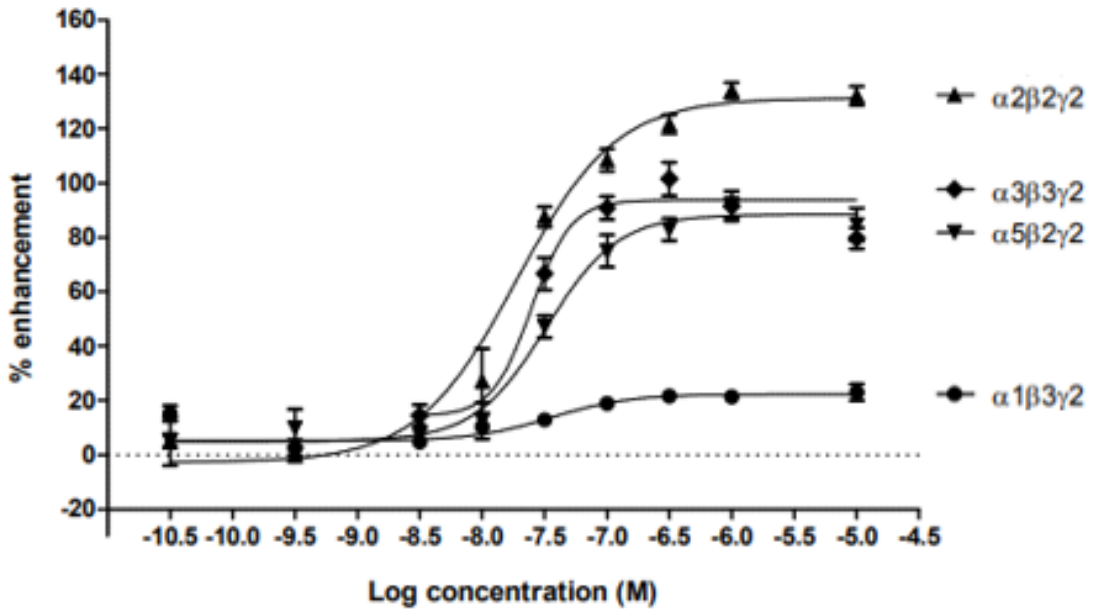
Abbreviations: CI = confidence intervals; EC = effective concentration; nM = nano molar; n.d. = not determined; SEM = standard error of the mean.

Modified from Nickolls et al., 2018. The *in vitro* binding and functional profile of darigabat were measured as described in Nickolls et al., 2018. Binding affinity (K_i) is shown in nM with geometric mean and 95% CIs. Functional activity is presented as mean ± SEM potentiation of a GABA EC_{10} concentration.

The functional activity of darigabat was determined in electrophysiology experiments in recombinant cell lines expressing $GABA_A$ receptors containing specific α subunits (Nickolls et al., 2018). The data generated determined that darigabat exhibited functional subtype selectivity for $GABA_A$ receptors containing α2, α3, and α5 subunits, with significant positive allosteric modulation (90–140%), but negligible activity (neutral modulation ≤20%) at $GABA_A$ receptors containing α1 subunits (**Figure 1-3**). Therefore, although the higher affinity for darigabat at α1-containing receptors will result in greater RO at these receptors compared to receptors containing α2/α3 or α5 subunits (for the

given dose), this should result in little functional effect because of the very low level of allosteric modulation.

Figure 1-3 Functional activity of darigabat in recombinant cell lines



Functional selectivity of darigabat for α2/3/5 $GABA_A$ receptors subunits and minimal functional activity at α1 subunits. Concentration-response curves of QPatch functional response for human $GABA_A$ receptors containing different α subunits, graphic taken from Nickolls et al., 2018. Mean ± standard error of the data from all experiments (n=3-7).

Due to its favourable *in vitro* profile which met the pre-defined in-house requirement of approximately <20% α1 potentiation and >50% α2 potentiation, darigabat was progressed to *in vivo* experiments (see **Chapter 2**). These studies were designed to confirm whether the proposed benefits of an α1-sparing profile translated to the ability to achieve high RO without dose-limiting side effects, and to efficacy in a range of nonclinical models of anxiety, epilepsy, and pain.

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2 BIOMARKERS

2.1 Introduction

Attrition in the pharmaceutical industry has been a long-standing challenge. Long timelines and high failure rates make drug development for neurological disorders a particularly expensive and risky business. On average across different clinical indications, it takes approximately 10 years from the start of Phase 1 clinical trials to approval by a regulatory agency, and the cost to develop a new molecular entity (NME) is on average $2.6 billion (Kaitin and DiMasi, 2011). The success rates in neuroscience are lower than for any other therapeutic area, with only 8% of products that initiate clinical development being subsequently approved (compared to 15% across all indications), a rate potentially reflective of the complexity of the neurological syndromes with multiple etiologies, and difficult to measure endpoints. In recent years many large pharmaceutical companies, including Pfizer and GlaxoSmithKline, have made the difficult decision to cease working on neuroscience research following thwarted efforts to develop new, safe, and effective drugs to treat pain, anxiety, neurodegenerative diseases, and other neurological indications. The stark realities of drug development have highlighted that there is a need to do more in the way of translational research, and a need to identify ways to increase the probability of technical and regulatory success and stop early if the data indicates it is warranted.

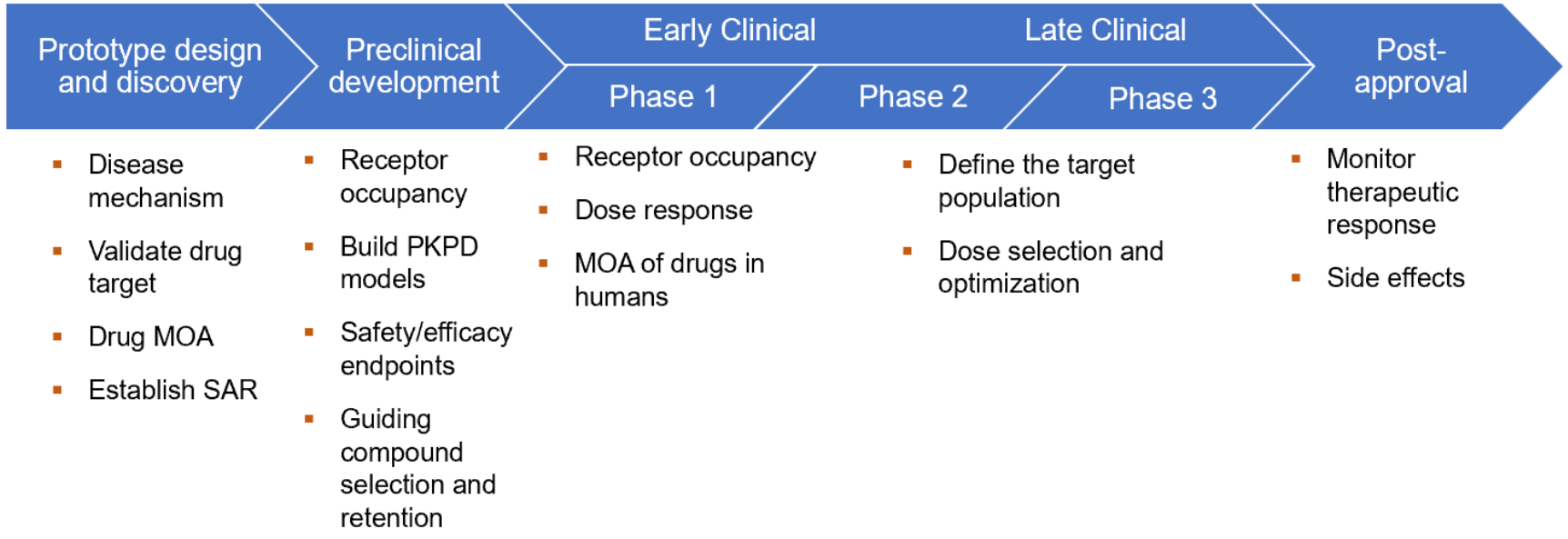
Some progress however has been made in certain research disciplines to identify those NMEs that are unsuitable to progress into clinical development. During 1991, poor pharmacokinetic (PK) and/or bioavailability properties of drugs in early clinical development was the largest cause of attrition, being responsible for a hefty 40% of failures (Kaitin and DiMasi, 2011). Less than 10 years later, this cause of attrition had reduced to 10%, a success credited to the successful adoption of the “rule of five” proposed by Chris Lipinski who had observed that approved drugs had typical physiochemical and structural properties, including for example molecular weight of less than 500 Daltons (Lipinski et al., 1997). Whilst this did represent a significant step forward in the selection of drug candidates with more suitable clinical properties, there continues to be a significant proportion of drugs that continue to fail due to safety (30%), or efficacy

(at least 30% in CNS disorders), and ways in which to “fail fast, fail early” are sought to enable efficient decision-making in drug discovery.

Biomarkers are being increasingly used as part of the drug development toolkit to enhance decision making and reduce attrition. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, a pathological process, or a biological response to a therapeutic intervention. Biomarkers can be molecular (often termed “wet” or “fluid biomarkers”, for example levels of misfolded proteins in cerebrospinal fluid), histologic (e.g., changes in neuronal connectivity), radiographic (e.g., stroke lesion size) and/or physiologic (e.g., changes in electroencephalographic (EEG) activity), and can often be used nonclinically and clinically in both healthy participants and patients. They offer a potential opportunity to increase confidence in rationale or stop the novel compound/study early if the observed effects do not reach a pre-specified magnitude.

Several pharmaceutical companies have implemented biomarker strategies into the research and development continuum in the quest to identify new and effective therapeutics more efficiently (Morgan et al., 2012; Cook et al., 2014; Dolgos et al., 2016). They can be utilised in several different ways in early stages of drug discovery and development, including to understand the relationship between the chemical structure of a molecule and its biological activity (structure-activity relationship), to ensure drug exposure at the target site of action, that the drug binds to the pharmacological target, and to confirm expression of pharmacology. Biomarkers can bridge the gap between nonclinical data and patient populations in early clinical development, through dose ranging studies and right through to post-marketing surveillance of therapeutic efficacy and safety monitoring (**Figure 2-1**).

Figure 2-1 Utility of biomarkers throughout drug discovery and development



Abbreviations: MOA = mechanism of action; PKPD = pharmacokinetic/pharmacodynamic; SAR = structure-activity relationship.

In the darigabat program, the biomarker strategy has played a significant and valuable part in decision-making and has been used multiple times and at multiple stages throughout the program (**Figure 2-2**): to understand the mechanism of action (MOA), to confirm desired SAR in early discovery and to ensure that the drug concentrations in the CNS and the resultant pharmacology of darigabat was differentiated from benzodiazepines (BZDs) both nonclinically and in the clinic. Where biomarkers have not been used seamlessly in decision-making, they have helped to put into context negative data such as that observed in a clinical trial in pain (Gurrell et al., 2018, see **Chapter 3**). The biomarkers selected for use in the darigabat program and the associated results and role in decision-making will be discussed in the following Chapters. The contribution of the author to these works is outlined in **Table 1-1** in **Chapter 1.**

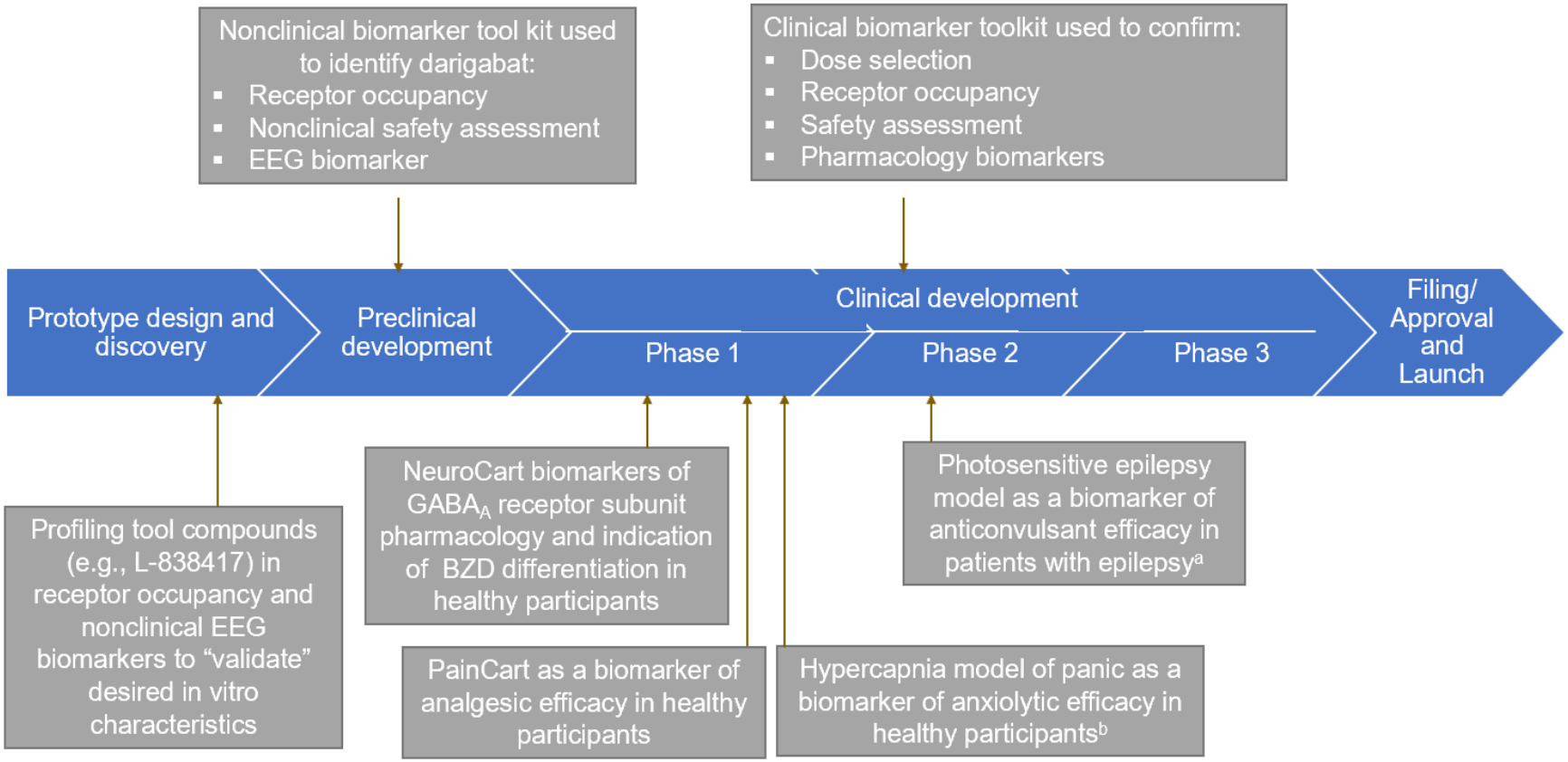
2.2 Biomarker studies

2.2.1 Receptor occupancy (RO)

The binding of a biotherapeutic to its cellular target can be measured quantitatively using displacement of a ligand in order to determine receptor occupancy (RO). Data generated from RO studies can be used to understand target specificity, PK, and pharmacodynamics (PD), and to model what predicted dose levels and administration schedules will lead to predicted levels of occupancy of a therapeutic agent. Collectively these measurements may help us understand or confirm the *in vivo* binding, concentration relevant to achieve desired biology to test the MOA and also to identify any potential safety concerns related to a specific dose or exposure of a compound. There are various types of RO assays that can measure different parameters of the drug-receptor interaction, and the selection of the assay format is driven largely by the MOA as well as the availability of labelled reagents.

The occupancy of brain $GABA_A$ receptors can be measured using [^{11}C]flumazenil, (a radiolabelled BZD site antagonist ligand) in animals and in healthy participants (Malizia et al., 1996). In rodents, $GABA_A$ RO is measured by pre-treatment with the compound of interest followed by intravenous administration of [^{11}C]flumazenil, shortly after which the animals are sacrificed, the brain is collected (along with the spinal cord in

Figure 2-2 The use of biomarkers in the darigabat research and development program



Abbreviations: BZD = benzodiazepine; EEG = electroencephalogram.

Diverse biomarkers have been used from early drug discovery to clinical development in the darigabat program.

[a] The clinical trial in patients with epilepsy is discussed in the Chapter 3.

[b] The clinical trial in hypercapnia model of panic has been initiated but is not yet complete and is discussed in **Chapter 5**.

specific instances), and levels of radioactivity counted. Clinically (and in nonhuman primates), $GABA_A$ receptors are readily imaged using [^{11}C]flumazenil using positron emission tomography (PET), and this technique has been shown to correlate well with measurements taken from brain tissue in rodents (Atack et al., 2011). However, given that flumazenil non-selectively binds to α1/2/3/5-containing $GABA_A$ receptors, the RO data generated with a compound reflects total ligand-binding site occupancy and does not provide information on binding to specific α subunit-containing receptors or intrinsic pharmacological activity. As such, interpretation of RO levels must be made in concert with other data such as behavioural pharmacology to establish the drug concentration-efficacy relationship.

Early in this drug discovery program rodent RO data was key in confirming that high occupancy can be achieved with α1-sparing compounds without significant impairment or sedation. This "proof-of-principle" utilised α2/3-selective tool compounds, L-838417 and TPA023, to develop an understanding of how the *in vitro* functional selectivity and intrinsic efficacy contribute to the pharmacology of the drug (Nickolls et al., 2011). If only low RO could be achieved before the emergence of significant impairment, it would have put into question the overall hypothesis that α1-containing $GABA_A$ receptors are the main contributor to the sedative side effect induced by BZDs. The data demonstrated that L-838417 and TPA023 were able to achieve maximal RO (see Figure 1 in Nickolls et al., 2011) and, via indirect measures of sedation utilising contralateral paw withdrawal thresholds in rats, this could be achieved without any impairment to the animal. This data generated with subtype-selective $GABA_A$ receptor modulators is supportive of the proposed contribution of α subunits to BZD pharmacology garnered from molecular studies, in which the $GABA_A$ receptor containing specific α subunits have been rendered unresponsive to diazepam in knock-out mice (Rudolph et al., 1999). However, clinical data generated subsequently did highlight a lack of concordance between the nonclinical profile and clinical profile with respect to sedation with some subtype-selective PAMs (Atack, 2010), which will be discussed later.

Having confirmed that high occupancy was possible with α2/3-selective tool compounds without significant impairment nonclinically, RO became a key component of the

screening sequence for NMEs identified from medicinal chemistry activities with the desired *in vitro* functional efficacy profile. Darigabat was profiled in mice, rats, nonhuman primates, and in healthy participants in the clinic (Nickolls et al., 2018). In rodents, darigabat demonstrated dose- and time-dependent plasma exposure and *in vivo* occupancy in brain, spinal cord, and cerebellum following oral dosing, with maximum total RO of > 80% in each tissue (see Figure 3 in Nickolls et al., 2018). The nonclinical occupancy data was used to select doses utilised in subsequent *in vivo* studies including zolpidem drug discrimination and EEG models. Predicted RO from plasma exposure in these studies was subsequently used to aid interpretation of the results. Furthermore, the nonclinical data (together with data generated in the first in human trial) were used to inform dose selection in the clinic.

In the clinical PET trial in healthy participants, approximately 1.5 hours following oral administration of single doses of darigabat at 10 and 65 mg, the median total $GABA_A$ RO in the whole brain was 68.6 and 88.9% respectively (**Table 2-1**; see Figure 9 in Nickolls et al., 2018). Given that the whole brain PET occupancy measure is α-subunit agnostic, a modelling-based approach was used to estimate the proportion of α1, α2, and α3-subunit containing receptors that were occupied by darigabat clinically. Specifically, this model used the *in vitro* binding affinities at α1/2/3 receptors and their relative abundance in human brain (for example, approximately 50% of $GABA_A$ receptors contain α1 subunits, McKernan and Whiting, 1996) to estimate RO in the 3 $GABA_A$ receptor subunit populations, with up to 80% RO achieved at α2-containing receptors (**Table 2-1**). The understanding of occupancy, particularly following the read out of proof-of-concept studies in pain (Gurrell et al., 2018) and anxiety (Simen et al., 2019), has helped refine the hypothesis that in fact it is likely that > 50% RO is required with subtype-selective PAMs with low intrinsic functional activity to elicit sufficient pharmacological effect.

Table 2-1 Observed and estimated receptor occupancy of oral doses of darigabat in humans

Darigabat oral dose	Observed maximum total $GABA_A$ RO (%)	Estimated maximum total $GABA_A$ RO (%)	Estimated maximum α2 subunit $GABA_A$ RO (%)
2.5 mg BID		60	25
10 mg SD	70		40
7.5 mg BID (or 17.5 mg SD)		80	60
25 mg BID (or 52.5 mg SD)		85	80
65 mg SD	90		80

Abbreviations: BID = twice daily; RO = receptor occupancy; SD = single dose.

Observed total receptor occupancy from positron emission tomography (PET) RO study examining single doses of darigabat (10 or 65 mg) at 1.5 hours post-administration. The relationship between $GABA_A$ RO in whole brain and plasma concentrations of darigabat can be well described by a simple Emax model (see Nickolls et al., 2018). Estimated total RO based on Emax model at expected plasma concentrations at that specific dose. Further modelling was conducted to estimate the receptor occupancy at α2 subtype receptors using the in vitro binding affinities of darigabat at α2 receptors and the relative abundance in human brain (Nickolls et al., 2018).

2.2.2 Electroencephalography (EEG)

Whilst RO and PET tracers such as [^{11}C]flumazenil can be used to confirm central penetration and target engagement of drugs, such studies do not confirm that drug binding results in physiological activity. However, EEG, which can be measured non-invasively, can be used as a "window" into brain function and a biomarker of central

pharmacology. The electroencephalogram is generated by inhibitory and excitatory postsynaptic potentials in cortical neurons. Scalp electrodes (used both nonclinically and clinically) record the summated postsynaptic potentials from the underlying cortex and can be used to examine the effects of drugs on sleep and on the power spectra. Normal humans sleep for consolidated periods at specific times of day whereas rodents do not, displaying instead an active period (dark phase) with a preference for wakefulness and an inactive period (light phase) with a preference for sleep. Despite this key difference, the ultradian (REM-NREM) pattern in rodents is similar to that in humans, although the cycles are much more rapid, and hence sleep changes have been proposed as nonclinical to clinical translational PD biomarkers for a range of mechanisms, including BZDs (Doran et al., 2008; Ivarsson, 2009; Leiser et al., 2011; Paterson et al., 2011). Polysomnography (sleep) studies in humans can be relatively expensive, with the general requirement that participants stay at a clinical research unit or hospital overnight, ideally for several nights to ensure they are acclimatised to the new conditions, in order to examine the effect of a drug on sleep. However, the effect of a drug on power spectra recorded from EEG (or quantitative EEG (qEEG)) can be measured over a short duration when a person is awake.

Power spectral analysis is a standard method for the quantification of EEG with mathematical processing of digitally recorded EEG to discriminate between specific waveform components. The power spectrum reflects the distribution of signal power over specific frequencies and is split into 5 categories of frequency bands typically defined as delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-100 Hz) (Steriade et al., 1990). CNS penetrant drugs can have effects on sleep and/or the power spectra, and drugs used to treat distinct CNS disorders have been shown to have distinct signatures on qEEG in rodents (Dimpfel, 2003). Non-selective BZDs have been shown to contribute to the sleep-wake cycle and increase β, γ, and δ power, and decrease α and θ power, and α1-selective agonists (such as zolpidem) primarily increase δ power (Tan et al., 1998; Lancel and Steiger, 1999). Furthermore, qEEG studies have demonstrated that the diazepam-induced increases in β frequency remains unaffected in α1 or α3 knock-in

mice but is reduced in mice with diazepam-insensitive α2 subunits (Tobler et al., 2001; Kopp et al., 2003; Kopp et al., 2004).

This key data led to an exploration of the potential to use β power as measured by recording EEG in freely moving rats as confirmation of α2-target engagement using α2/3-selective tool compounds L-838417 and TPA023 (Nickolls et al., 2011). Rats under anaesthesia were surgically implanted with radio telemetric transmitters for the recording of EEG and electromyogram (EMG) and were allowed to recover for at least 2 weeks prior to use and only after confirmation they were healthy to continue following evaluation by a veterinary surgeon. In two separate experiments rats were treated with vehicle and 3 different doses of compound in a cross-over design, thus enabling within animal comparisons, and EEG and EMG were continuously sampled at 500 Hz from the point of dosing until approximately 24 hours later. The three doses of each tool compound selected for the qEEG evaluation were based on RO, and targeted approximately 50, 75, and 100% occupancy (Nickolls et al., 2011). For qEEG analysis, every 12 second epoch was Fast Fourier transformed and the absolute power within each of the five frequency bands was calculated.

Pharmacological activity was observed with both L-838417 and TPA023. L-838417 dose-dependently increased β frequency and there was a trend to increase β frequency with TPA023, although this was not statistically significant, correlating with the lower intrinsic efficacy at α2 of TPA023 compared with L-838417 (see Figure 8 in Nickolls et al., 2011). As such, changes in qEEG β frequency was proposed as an appropriate pharmacological biomarker for α2-selective $GABA_A$ PAMs, and complemented RO in the screening cascade as part of the nonclinical biomarker toolkit to identify suitable NMEs for further research.

Darigabat was selected to be investigated in a rodent qEEG study following demonstration of appropriate *in vitro* attributes and *in vivo* RO. The study design was similar to that used previously with tool compounds L-838417 and TPA023, and doses of 1, 3, and 10 mg/kg administered orally were selected to cover a predicted RO range of 30-80%. Darigabat was observed to increase β frequency dose-dependently in rodents, confirming the desired frequency “signature” on qEEG of a selective-$GABA_A$ receptor

PAM (see Figure 4 in Nickolls et al., 2018), and was therefore deemed suitable to move forward into nonclinical efficacy and toxicology studies (see **Chapter 4.2.1**). Furthermore, based on the translatability of qEEG from nonclinical studies to the clinic, it was selected as a biomarker assessment parameter as part of a first-in-human (FIH) trial which is described next.

2.2.3 NeuroCart in the first-in-human clinical trial

FIH studies are traditionally used to understand single dose PK and the safety and tolerability profile of the drug under investigation to identify whether it is suitable for continued clinical development through an anticipated dose range. FIH trials with other $GABA_A$ receptor PAMs with desired nonclinical α1-sparing properties have previously revealed unexpected adverse observations related to sedation and drowsiness in the clinic (Atack, 2010). For example, MK-0343 is reported to exhibit α3 > α2 = α5 > α1 subtype-selectivity, with low intrinsic efficacy of the latter (~20% of diazepam) yet dose-limiting sedation was observed in the clinic at levels of RO below the limit of detection (<10%; de Haas et al., 2008), and insufficient to progress into further efficacy exploration. Subsequent compounds (e.g., TPA023 and TPA023B), which exhibited lower functional efficacy at α1 subunits than MK-0343, also possessed dose-limiting adverse events (AEs) including drowsiness which precluded dosing beyond approximately 70% RO (Atack, 2010). Given that darigabat exhibited no known differentiable nonclinical attributes from, for example, TPA023, it was unknown whether the clinical properties of darigabat would be differentiated from those observed with TPA023.

Beyond the traditional PK, safety, and tolerability objectives, the FIH can also be an opportunity to assess PD endpoints that are indicative of the drug accessing the target tissues (e.g., the brain), and confirm expression of target-specific pharmacology. By the time that darigabat was ready to enter the clinic for the first time, assessment of biomarkers of non-selective BZDs was well established, with more than 170 PD tests or test variants developed to assess their CNS effects (de Visser et al., 2003). De Visser and colleagues analysed cross-trial reproducibility, sensitivity, and specificity of the frequently used biomarkers and identified saccadic peak velocity (SPV) and the visual analogue scale (VAS) of alertness as the most sensitive parameters for BZDs, with both

endpoints showing consistent effects of dose ranges for several BZD compounds. In the last 20 years these individual endpoints have been combined into a battery of tests by the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands, who have developed and utilised a "-Cart" concept for neurological and pain testing using rapid, multifunctional, automated data capture systems for pharmacodynamic/pharmacokinetic (PKPD) assessment in early clinical development trials. Their proprietary NeuroCart is a battery of CNS evaluations that can be administered at multiple timepoints prior to and following administration of drug and have been used extensively to investigate the effects of various CNS-active compounds where they have been shown to be sensitive to detect the effects of subtype-selective $GABA_A$ receptor PAMs (Chen et al., 2012). The constituent parts of the test battery target a variety of neurophysiological and/or neuropsychological domains (**Table 2-2**). Comparative effect profiling, as utilised in these studies, can be used to provide an indication of the pharmacological selectivity and specificity of novel GABAergic modulators (de Haas et al., 2007). As such, reduction of SPV has been proposed to be related to α2/3 subunit-mediated anxiolytic effect of BZDs (de Visser et al., 2003), although it is also reduced during sedation (van Steveninck et al., 1999). Body sway could be influenced by α2-subunit mediated muscle relaxation or α1-mediated reduction in alertness, although given that α1-preferring zolpidem causes postural instability (Mattila et al., 1998), is suggestive of the involvement of α1 > α2-containing receptors. Adaptive tracking is a task that measures drug effect on visuomotor coordination and has been shown to be sensitive to the sedative effects of sleep deprivation (van Steveninck et al., 1999), affected by reduced alertness and impaired by zolpidem (Mattila et al., 1998), implicating α1 subunit-containing receptors playing a role. Effects on memory have implicated the α5 subunit (Collinson et al., 2002), although effects on alertness via α1 cannot be ruled out. qEEG can also be included to confirm the drugs frequency "signature" and provide further confirmation that drug exposure is in the correct compartment and exposure range. Thus, NeuroCart endpoints have the propensity to quantitatively detect both the desired α2/3-mediated effects of a $GABA_A$ receptor PAM, but also the undesired α1/5-mediated effects to enable comparisons with

BZDs and facilitate understanding of PKPD early in drug development, particularly when combined with a FIH dose-ranging trial.

Table 2-2 Example constituent tests of the NeuroCart battery, related CNS domains, $GABA_A$ receptor pharmacology and hypothesised effect of an α2/3/5-subtype selective $GABA_A$ receptor PAM

NeuroCart test	Targeted domain	$GABA_A$ pharmacology	Hypothesised α2/3/5-subtype selective $GABA_A$ PAM profile
Saccadic peak velocity	Neurophysiological function	α2/3-subunit selective effect	Dose-related desired α2/3-subunit target engagement, effect size surpasses BZD
Body sway	Balance	Predominantly α1-subunit mediated effect	Minimal impairment compared to BZD
Adaptive tracking	Visuomotor coordination	Predominantly α1-subunit mediated effect	Minimal impairment compared to BZD
Visual verbal learning test	Memory	α1/5-subunit mediated effect	Minimal impairment compared to BZD
VAS Bond and Lader	Alertness, mood, calmness	Non-selective effect	Minimal impairment compared to BZD
Quantitative EEG	Neurophysiological function	α2/3-subunit mediated increases in β frequency band α1-subunit mediated increases in δ frequency band	Increase in β frequency band predominate, minimal increase in δ frequency band

Abbreviations: BZD = benzodiazepine; EEG = electroencephalogram; PAM = positive allosteric modulator; VAS = visual analogue scale.

Accordingly, the single ascending dose FIH study was designed to examine PK, safety and tolerability, and PD effects of darigabat using NeuroCart in healthy participants and is described in detail in Nickolls et al., 2018. The doses explored in the trial ranged from 0.04 mg to 100 mg in four cohorts, with the fourth cohort including lorazepam 2 mg to enable comparisons of PD effects with an established non-selective BZD positive control

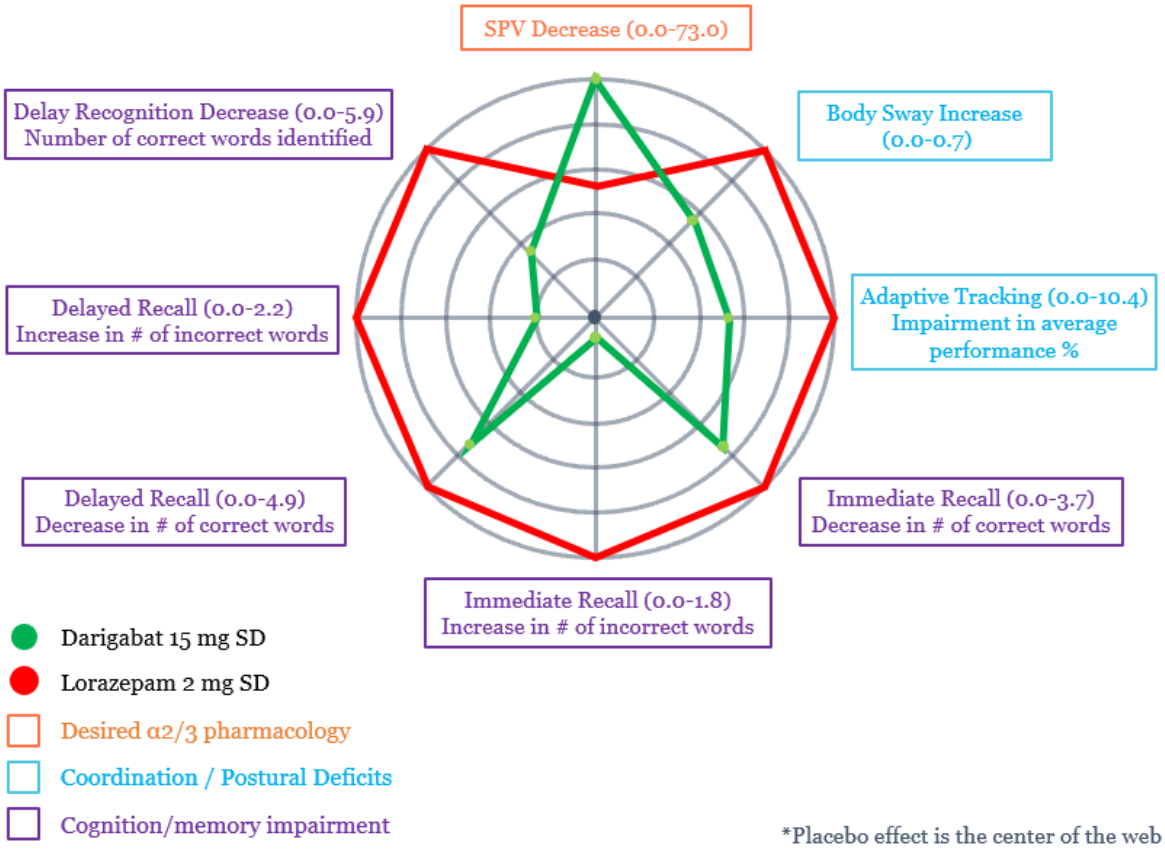
molecule. All doses were administered as on oral suspension except for one group in which 25 mg was also administered as a tablet formulation to investigate relative bioavailability between the oral suspension and tablet formulation. NeuroCart assessments were performed at pre-dose and at 0.5, 1, 1.5, 2, 4, 6, and 12 hours post-dose. A total of 45 participants completed the trial. Darigabat was absorbed rapidly with median T_{max} of 1-4 hours, and mean $t_{1/2}$ of 6-9 hours and in general the systemic exposure appeared to increase proportionally across the entire dose range examined (see Table 3 and Figure 6 in Nickolls et al., 2018). All doses of darigabat were safe and well tolerated, with the incidence and severity of adverse events (AEs) not increasing with escalating doses beyond 6 mg. A maximum tolerated dose was not demonstrated with darigabat, in contrast to, for example, TPA023 (Atack, 2010), establishing that although the nonclinical profiles were approximately similar, the clinical profile was divergent.

NeuroCart data demonstrated that doses of 4 mg and higher of darigabat were related to dose-dependent, statistically significant reduction in SPV versus placebo (see Figure 8 in Nickolls et al., 2018). The effects of doses > 50 mg of darigabat were similar, with a reduction in the SPV averaged over the first 6-hour post-dose (based on the PK properties of darigabat) of approximately 130 degrees/sec, compared to approximately 40 degrees/sec with lorazepam 2 mg. In body sway, the effects of dosing darigabat at 0.8 and 4 mg and the majority of the higher doses were statistically significantly greater than placebo. The effects on body sway at doses ranging from 10 to 100 mg were approximately similar at ~ 1.55 ratio versus placebo, by comparison lorazepam 2 mg demonstrated a greater increase in body sway versus placebo at 1.97. In qEEG, there was a statistically significant increase in β power and decreases in δ and θ power at doses at 15 mg and above (see Figure 8 in Nickolls et al., 2018). Administration of lorazepam 2 mg elicited increases in β and decreases in α and θ power.

To date, further NeuroCart endpoints including adaptive tracking, visual verbal learning test (VVLT), and subjective effects measured with Bond and Lader visual analogue scales are yet to be published. However, adaptive tracking and VVLT have been presented at conference platform presentations (e.g., Gurrell, Epilepsy Pipeline Meeting, February 2018; Gurrell, Eilat XV, July 2020). In summary, darigabat 15 mg demonstrated less

impairment than lorazepam 2 mg in adaptive tracking and on measures of cognition (**Figure 2-3**).

Figure 2-3 Pharmacodynamic profile of single oral doses of darigabat 15 mg and lorazepam 2 mg on NeuroCart endpoints of saccadic peak velocity, body sway, adaptive tracking, and immediate and delayed recall



Abbreviations: mg = milligram; SD = single dose; SPV = saccadic peak velocity.

Spider plot represents contrasts per treatment per domain compared with placebo. Distal from the centre indicates Least Square Mean estimation greater than placebo.

The totality of the clinical safety and the PD data with darigabat is aligned with the expected pharmacology of an α2/3 selective $GABA_A$ receptor PAM with minimal activity at α1. Importantly, it shows differentiation on predominately α1-mediated effects from a dose of lorazepam therapeutically used to treat anxiety. The body sway endpoint

assessing postural stability was the most sensitive PD endpoint with small, but significant increases occurring at sub-milligram doses. These increases plateaued at approximately 10 mg and did not further increase up to 100 mg despite marked increases in RO across this dose range. This apparent sensitivity could be related to the fact that although darigabat has minimal functional efficacy at α1, it has a K_i of 0.18 nM at α1-containing receptors, 16-fold lower than at α2 (see Table 1 in Nickolls et al., 2018). Thus, the α1-containing receptors will be preferentially occupied at lower doses, with significant α2 occupancy occurring at relatively higher doses. Despite this, the impact on body sway (and adaptive tracking) was less than elicited by lorazepam, potentially due to the limited functional efficacy possessed by darigabat at α1 subtypes, indicating that darigabat has considerably less impairment on postural coordination than a non-selective BZD.

The assessment of qEEG in this clinical trial complements the nonclinical studies conducted in rats with other subtype-selective PAMs and darigabat (Nickolls et al., 2011; Nickolls et al., 2018). Comprehensive analysis of the effects of zolpidem, lorazepam, and numerous subtype-selective $GABA_A$ receptor modulators, including TPA023 and AZD7325, concluded that it is indeed α2/3 activity that correlates with an increase in β and γ power in spectral analysis (Christian et al., 2015). The current pharmacological tools do not permit the relative contributions of α2 and α3 subunits to be dissociated; however, previous work with diazepam-insensitive mutant mice indicates that the α2 subunit is primarily responsible for modulating these effects (Kopp et al., 2003; 2004). The clinical data obtained with darigabat support the use of qEEG as a translatable biomarker for $GABA_A$ receptors given that the nonclinical changes of α, β, θ, and δ power observed in qEEG of rats treated with darigabat were correlated with similar changes in the qEEG in the darigabat-treated healthy human participants.

Effects on SPV are thought to be related to anxiolytic properties of BZDs (de Visser et al., 2003). A review published by de Visser and colleagues found that SPV dose-dependently increased across the studied temazepam (a BZD approved for the treatment of anxiety) doses in anxiety and used this analysis to examine dose equivalences for six additional BZDs. They proposed that a minimal effective dose for treatment of anxiety could be predicted based on the SPV change being equivalent to that induced by the lowest dose

of temazepam that is effective in anxiety. A significant correlation was observed which suggested that concentrations of a drug which induced a reduction of SPV of at least 50 degrees/sec had the potential to be anxiolytic (de Visser et al., 2003). Data observed with two subtype-selective PAMs concur with this proposed predictive biomarker model. TPA023 concentrations achieving ~70% RO was found to affect SPV to a similar degree as 2 mg lorazepam (de Hass et al., 2007) and has been shown to have potential anxiolytic activity in generalised anxiety disorder (GAD; Atack, 2009), whilst AZD7325 at ~80% RO demonstrated a smaller reduction in SPV than that observed with lorazepam 2 mg (Chen et al., 2014) and was subsequently found to ineffective in GAD (CT.gov ID NCT00808249). The robust reduction of SPV observed with darigabat was approximately equivalent to lorazepam 2 mg at only 4 mg of darigabat (~30% RO) and twice that of lorazepam at the 65 or 100 mg doses (> 80% occupancy). As such, together with the nonclinical knockout data purporting the α2/3 subunit containing receptors are responsible for the anxiolytic activity of BZDs (Rudolph et al., 1999), it raised the potential possibility that relatively low RO of darigabat had the potential to be anxiolytic.

The NeuroCart trial discussed here was a hypothesis generating FIH clinical trial designed to guide decision-making and explore the PKPD and safety of darigabat in healthy participants using a CNS test battery. Data generated using NeuroCart established a good correlation between the PKPD profile of darigabat in healthy participants with both the nonclinical binding and functional selectivity profile. The data was used to select doses of darigabat in subsequent clinical trials and enabled interpretation of the trial data in the context of the PD profile in NeuroCart, more of which will be discussed in **Chapter 3**. Overall, the PK, safety, and PD profile demonstrates that darigabat possesses desired attributes that correlate with the proposed MOA of enhancing GABAergic modulation through selectively targeting α2/3-containing receptors, with minimal impact at α1-containing receptors, making it an ideal compound with which to study the potential benefits of subtype-selective $GABA_A$ receptor PAMs.

2.2.4 PainCart

Human pain models are useful for assessing potential analgesic effect of drugs and have been used extensively in early clinical research for many years. Models have been

developed and utilised to establish whether a drug is acting peripherally or centrally, and whether it is suitable for a particular pain modality, i.e., nociceptive, inflammatory, or neuropathic. Once a suitable stimulus and endpoint has been established, it can be used in combination with PK which may aid future dose selection. However, given the complex nature of clinical pain, a failure in one experimental pain model does not preclude success in a different model examining a different aspect of pain, highlighting the need to use a comprehensive battery of pain models. In approximately 2012 CHDR began development of a PainCart concept similar to their well-validated NeuroCart platform. It was designed to enable the assessment of multiple functional pain endpoints to be tested before and multiple times after drug treatment, with the advantage over the single assays being the diversity of functional pain endpoints evaluated in a setting amenable to gain PKPD data from drug responses.

2.2.4.1 Phase 1 PainCart validation clinical trial

Following some personal communications with CHDR whilst discussing the darigabat FIH NeuroCart design, the concept of 'PainCart' was discussed with the objective of assisting early clinical research efforts to identify novel analgesics. Subsequently, a limited collaboration was established between Pfizer and CHDR to design and execute a PainCart validation trial using approved analgesics, in preparation for future potential studies with novel compounds in early clinical development. The pain models in the validation trial were selected to represent a broad range of pain modalities (heat, cold, mechanical, electrical, and sensitised) and nociceptor function (Okkerse et al., 2017), whilst utilising in-house experience of using the models at CHDR. The commonly used analgesics were selected to represent diverse mechanistic classes and were required to have previously shown activity in at least one pain model endpoint (**Table 2-3**). It was hypothesised that the battery of pain models would show distinct response patterns for the different analgesic classes. Furthermore, two different routes of administration of the analgesic (intravenous and oral) were selected to ensure flexibility for future studies with novel compounds.

Table 2-3 Overview of analgesic activity of varying mechanistic drug classes in different pain models

Drug	Stimulus				
	Electrical	**Cold pressor**	**Pressure pain**	**UVB+ Thermal**	**Thermal grill**
Strong Opioids (e.g., fentanyl)	Positive evidence[1]	Positive evidence[1]	Mixed evidence[1]	Positive evidence[1]	Positive evidence[2]
NMDA antagonist (e.g., ketamine)	Positive evidence[3]	Unknown	Positive evidence[3]	Unknown	Positive evidence[4]
NSAIDs (e.g., ibuprofen)	Positive evidence[3]	Mixed evidence[1]	Negative evidence[1]	Positive evidence[1]	Unknown
TCAs (e.g., imipramine)	Positive evidence[3]	Mixed evidence[3]	Positive evidence[5]	Unknown	Unknown
Sodium Channel Blocker (e.g., phenytoin)	Unknown	Positive evidence[6]	Unknown	Unknown	Unknown
α2δ ligands (e.g., pregabalin)	Positive evidence[3]	Negative evidence[3]	Unknown	Positive evidence[3]	Unknown

Abbreviations: NMDA = N-methyl-D-aspartic acid; NSAID = non-steroidal anti-inflammatory drug; TCA = tricyclic antidepressant; UVB = ultraviolet B

1. Oertel et al., 2013; 2. Kern et al., 2008a; 3. Staahl et al., 2009; 4. Kern et al., 2008b; 5. Enggaard et al., 2001; 6. Webb et al., 1998.

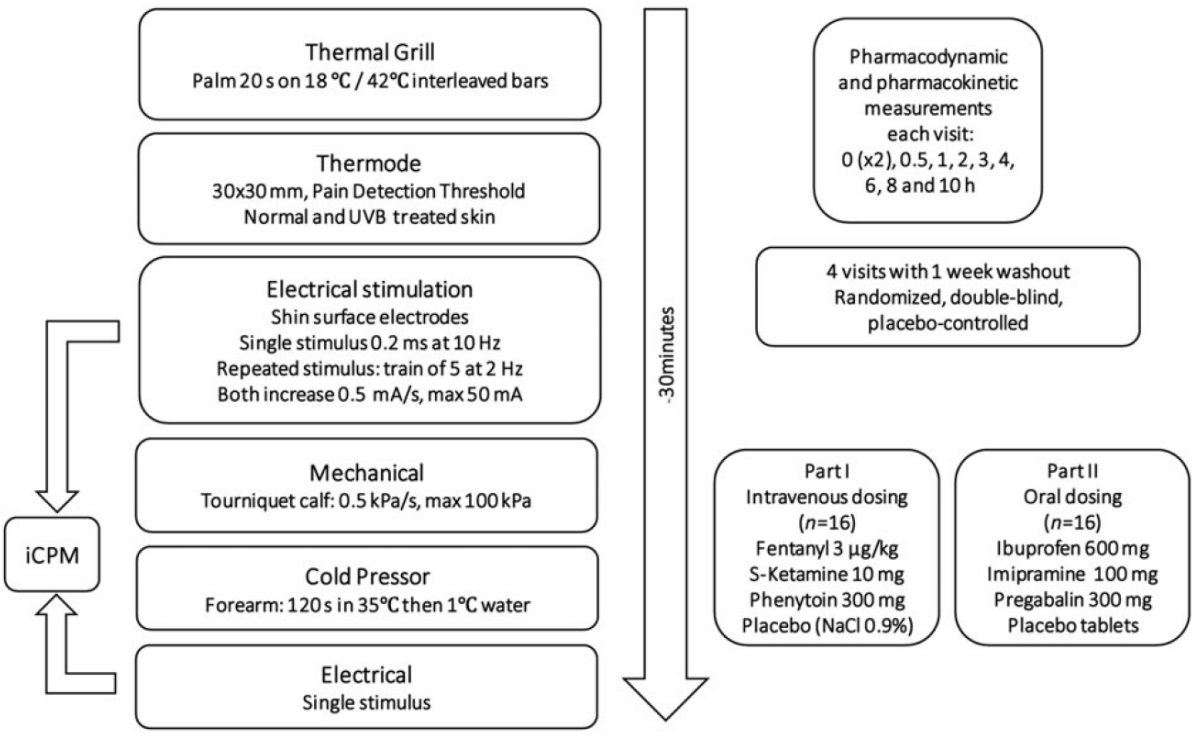
Evidence of analgesic activity of varying mechanistic drug classes in different pain models based on review of published literature. Adapted from Okkerse et al., 2017.

The PainCart validation trial was designed as a two-part, randomised, double-blind, placebo-controlled four-way crossover single dose trial in healthy male and female participants. In part 1 drugs were administered intravenously, and in part 2 they were administered orally. The total number of participants planned in each part was 16. In part 1, treatments consisted of intravenously administered fentanyl 3 µg/kg (strong opioid), phenytoin 300 mg (sodium channel blocker), (S)-ketamine 10 mg (NMDA antagonist),

and sodium chloride 0.9% (placebo). In part 2, treatments consisted of orally administered ibuprofen 600 mg (non-steroidal anti-inflammatory drug (NSAID)), imipramine 100 mg (tricyclic antidepressant), pregabalin 300 mg (ligand of α2δ-subunit of voltage gated calcium channels), and placebo tablet.

Nociceptive (pain) detection and tolerance thresholds were measured using a battery of integrated pain challenge tests with an aim of assessing as objectively as possible the levels of pain induced by several noxious mechanisms (described in detail in Okkerse et al., 2017). It took approximately 30 mins to complete the assessment of the PainCart test battery per participant, which were performed pre-dose and then at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours post-treatment administration (**Figure 2-4**). Blood samples were procured for drug concentration analysis in plasma at approximately the same time as the initiation of each PainCart battery assessment to examine the PKPD relationship of each drug and endpoint.

Figure 2-4 Overview of PainCart validation trial design



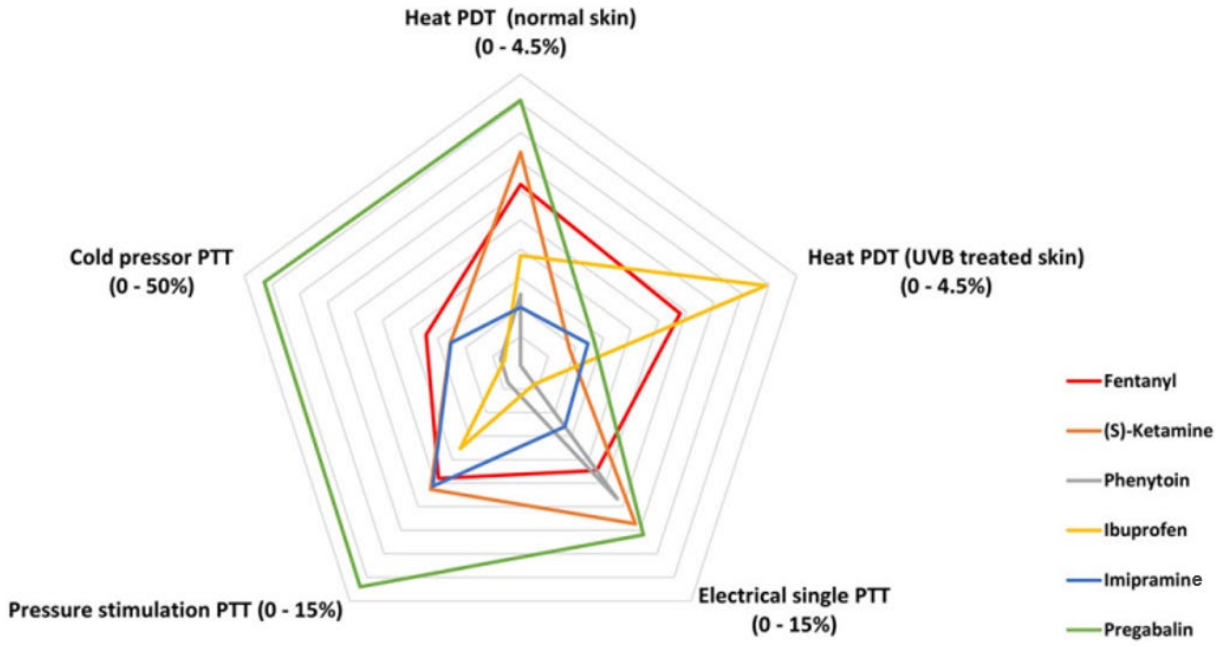
Abbreviations: iCPM = inhibitory conditioned pain modulation. Taken from Okkerse et al., 2017.

The results showed that each compound tested demonstrated its own profile of effects on evoked pain in the different models included in the PainCart test battery, see Figures 3-5 in Okkerse et al., 2017). For example, the strong opioid fentanyl, influenced a broad range of pain tests including effects on electrical pain, cold pressor, thermal pain, and thermal grill, and modulated pain thresholds in the cold pressor test and thermal testing, which is perhaps unsurprising given the broad use of strong opioids in different types of clinical pain. Of all the compounds administered, pregabalin showed the largest effect on most of the pain paradigms (heat, cold, pressure, and electrical pain), although this may be in part due to the selection and use of the 300 mg dose which is relatively high compared to how it is commonly used to treat, for example, neuropathic pain. The effect of ibuprofen reflected its inhibition of cyclooxygenase by this NSAID, demonstrating effectiveness in reducing heat hyperalgesia in skin sensitised with UVB and agrees with previously reported data (Oertel et al., 2013). A summary of the profile of each drug on the pain challenges are shown in **Figure 2-5.**

Although only one dose of each compound was used, the PD profiles of these single doses matched the plasma concentration profile of the compounds used. The analgesic profiles for the different mechanistic classes in the PainCart validation were comparable to those published previously (Webb et al., 1998; Enggaard et al., 2001; Kern et al., 2008a and 2008b; Staahl et al., 2009; Oertel et al., 2013).

In conclusion, it was shown that this battery of pain models is suitable to identify changes in pain detection and pain tolerance thresholds after administration of different classes of analgesic compounds in healthy participants. The analgesic compounds all showed a unique profile in their effects on the pain tests administered and these profiles were in most cases compatible with the expected pharmacology. The knowledge of these profiles can be used to benchmark analgesic properties of these new drugs against established analgesics in early phase clinical studies in healthy participants.

Figure 2-5 Spider plot of effect sizes of drugs used in PainCart validation trial



Abbreviations: PDT = pain detection threshold; PTT = pain tolerance threshold; UVB = ultraviolet-B.

Effect sizes are shown as the difference between each drug and placebo.

Taken from Okkerse et al., 2017.

2.2.4.2 Phase 1 PainCart clinical trial with darigabat

The PainCart trial with darigabat was initiated on 30th September 2014 and completed on 26th November 2014, which is testament to the possibility to generate data from well-controlled biomarker studies in a very short timeframe. The trial was a double-blind, single-dose, placebo- and active-controlled four-period cross-over trial in which the effects of 2 doses of darigabat (15 and 65 mg) were compared with placebo and pregabalin 300 mg (van Amerongen et al., 2019). Doses were selected based on achieving an effect on SPV greater than that observed with 2 mg lorazepam in the FIH trial and ~ 50% RO, and a higher dose that was expected to achieve maximal RO (~80%) and maximal PD effect on SPV. Pain thresholds were measured in twenty healthy adult male participants aged 18-55 years prior to dose administration and at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours post-treatment administration, as was performed in the validation trial. A

PainCart training session for participants was included as part of the screening eligibility examination to exclude non-responders or extreme responders.

The battery of evoked pain tasks consists of the following tasks for nociception: electrical stimulation task, pressure stimulation task, heat pain, and the cold pressor task (**Figure 2-4**). The test battery also includes a model for inflammatory pain (the UVB model), and a paradigm to quantify inhibitory conditioned pain modulation (iCPM). For the electrical stimulation task, the pressure stimulation task, and the cold pressor task, pain intensity was measured continuously (beginning from when the first stimulus was applied until the end of the test) using an electronic visual analogue scale (VAS) scale ranging from 0 (no pain) to 100 (most intense pain tolerable). For the above-mentioned pain tasks, the pain detection threshold (PDT), pain tolerance threshold (PTT), and a post-test VAS score were determined. For the thermal pain tasks (normal skin and UVB exposed skin), only the (average of triplicate) PDT was determined as assessment of heat PTT is prone to induce tissue damage. The primary endpoints of the study were as follows: thermal pain (normal skin) PDT; thermal pain (UVB-sensitised skin) PDT; electrical stair PTT; pressure pain PTT; cold pressor PTT. Secondary endpoints included iCPM response and post-test VAS scores. Blood samples were procured for drug concentration analysis in plasma at approximately the same timings as the initiation of the PainCart battery to examine the PKPD relationship.

Sample size and decision criteria were based on mean effect over the first 6 h after dosing for the primary endpoints: PTT for the pain tasks electrical pain, pressure pain, and cold pressor, and PDT for the thermal pain tasks (normal skin and UVB skin). The decision criteria applied to the trial was 95% confidence that the effect of darigabat was greater than placebo. Decision criteria were not applied to secondary endpoint in the trial.

A dose of 15 mg darigabat met the decision criteria and increased PTTs for pressure pain compared with placebo, whilst 65 mg darigabat led to an increase in PTT for the pressure pain task and cold pressor, meeting the decision criteria (**Table 2-4**). Decision criteria were not met by either dose of darigabat on thermal endpoints in normal skin or UVB-induced inflamed skin, or in the electrical stair. The magnitude of effect was greater for 65 mg (> 80% RO) compared with 15 mg (~ 60% RO) for the cold pressor, but effects

were similar for the pressure pain task. Pregabalin (300 mg) showed an increase in PTT for pressure pain and cold pressor task. The effect of pregabalin was greater than darigabat for both tasks (see Figures 1 and 2 and Table 2 in van Amerongen et al., 2019).

Table 2-4 Summary of decision criteria for pharmacodynamic primary endpoints in PainCart trial with darigabat

Primary Endpoint	Treatment	Comparison to placebo		
		Mean difference	90% CI	Probabilities associated with decision criteria[a]
Thermal pain (normal skin PDT)	Darigabat 15 mg	-0.143	(-0.780, 0.495)	0.36
	Darigabat 65 mg	-0.297	(-0.928, 0.333)	0.22
	Pregabalin 300 mg	0.214	(-0.412, 0.840)	n.d.
Thermal pain (UVB skin PDT)	Darigabat 15 mg	0.143	(-0.700, 0.985)	0.61
	Darigabat 65 mg	0.343	(-0.523, 1.209)	0.74
	Pregabalin 300 mg	0.383	(-0.454, 1.219)	n.d.
Electric stair (PTT)	Darigabat 15 mg	1.05	(0.96, 1.15)	0.81
	Darigabat 65 mg	1.09	(1.00, 1.20)	0.94
	Pregabalin 300 mg	1.05	(0.96, 1.15)	n.d.
Pressure pain (PTT)	Darigabat 15 mg	1.11	(1.02, 1.22)	**0.97**
	Darigabat 65 mg	1.11	(1.01, 1.21)	**0.97**
	Pregabalin 300 mg	1.15	(1.06, 1.26)	n.d.
Cold pressor (PTT)	Darigabat 15 mg	1.06	(0.94, 1.21)	0.79
	Darigabat 65 mg	1.17	(1.03, 1.32)	**0.98**
	Pregabalin 300 mg	1.31	(1.16, 1.48)	n.d.

Abbreviations: CI = confidence interval; n.d. = not determined; PDT = pain detection threshold; PTT = pain tolerance threshold; UVB = ultraviolet radiation B.

[a] At least 95% confident that darigabat effect was greater than placebo. Decision criteria were not calculated for pregabalin. Decision criteria were not applied to secondary endpoints.

The absence of anti-inflammatory effects of darigabat is perhaps surprising given that hyperalgesia resulting from cutaneous (UVB-induced) inflammation is known to be of peripheral origin (Treede et al., 1992), and the spinal α2 $GABA_A$ receptors are located and act at the spinal or supraspinal level (Knabl et al., 2008; Knabl et al., 2009; Paul et al., 2012). The lack of effect of darigabat on iCPM may be due to this trial being in healthy participants and this endpoint may be more susceptible to modulation in chronic pain states where there is GABAergic dysregulation (Knabl et al., 2008).

Overall, subtype-selective modulation of $GABA_A$ receptors by darigabat resulted in a distinct PD effect profile indicative of the analgesic potential of darigabat. The domains in which efficacy was observed overlapped with pregabalin, a drug approved drug to treat neuropathic pain and fibromyalgia. Quantitatively, the data showed 300 mg pregabalin > 65 mg darigabat > 15 mg darigabat, suggesting that even at high RO in this experimental medicine trial darigabat has potentially more modest analgesic potential than pregabalin. However, the quantitative relationship between endpoints in these experimental challenge models and clinical endpoints from patients in clinical trials has not been established. The data also highlights the importance of high RO to attain greater efficacy, with a more robust profile observed at 65 mg (~80% RO) compared to 15 mg (~60% RO) darigabat. Indeed, nonclinical studies in mice indicated that ~70% of α2 $GABA_A$ receptors need to be drug-bound (even when a high intrinsic activity ligand is used) to achieve a significant reduction in pain thresholds (Ralvenius et al., 2015).

Determining PD effects of novel drugs in healthy participants prior to moving to patient studies can reduce risk by establishing pharmacology and aiding in dose selection. Although the PainCart darigabat healthy participant trial has the potential to bridge that gap, it was in practice executed in parallel with a clinical trial of darigabat in patients with pain (Gurrell et al., 2018). As such, the PainCart trial was not used as decision-making to proceed into patients with pain. The reasons for executing both healthy participant and patient studies in parallel was multifactorial, including confidence in the nonclinical rationale; that PainCart had only been relatively recently established and validated; and the pressure of development timelines. Therefore, the PainCart healthy participant trial was used only to add to rationale of the analgesic potential of darigabat. As such, the

conclusions from this trial related to RO and the quantitative comparison to the positive control pregabalin or placebo were not considered in the design of the chronic low back pain trial. This is further discussed in **Chapter 3**.

2.3 Discussion

The darigabat program adopted a biomarker-based approach to enable understanding of target binding and expression of pharmacology both nonclinically and clinically (Nickolls et al., 2018). Using this approach, a lack of nonclinical *in vivo* α1 activity was determined using a rodent zolpidem drug discrimination model and the α2 activity was measured using qEEG which translated successfully from the nonclinical to the clinical setting. The NeuroCart battery utilised in the FIH trial included tests to measure both desired α2/3 pharmacology using SPV and qEEG, and undesired α1/5 pharmacology using body sway, adaptive tracking, and the VVLT. Overall, the data demonstrated that darigabat is generally well-tolerated across the entire dose-occupancy range and possesses minimal effects on undesired α1/5-mediated endpoints. Furthermore, it induces large functional effects on the α2/3-related PD endpoints, thus making it an ideal tool to further study the potential benefits of subtype-selective $GABA_A$ receptor PAMs.

A reduction in SPV, which is purportedly closely related to the anxiolytic properties of BZDs (de Visser et al., 2003) and driven by α2/3-containing $GABA_A$ receptors, demonstrated by darigabat was approximately three times that of 2 mg lorazepam at the 65 and 100 mg doses. This magnitude of SPV reduction, likely related to both the high levels of α2 RO that can be achieved without dose-limiting adverse events and the relatively high level of efficacy this compound possesses at α2-containing receptors, was a contributing factor in the subsequent decision to conduct a clinical trial in patients with anxiety, which is discussed in **Chapter 3**.

PainCart was established and validated in a collaborative effort to have available a tool with which to benchmark analgesic properties of new drugs against established analgesics in early phase clinical studies in healthy participants (Okkerse et al., 2017). Given the large number of pain models used in PainCart, the even larger number of outcome variables yielded, and the lack of correction for multiple testing, it was designed

to be used as a screening tool for analgesics and not to definitively prove effects on a specific evoked pain model with significance. Darigabat had an analgesic effect on pressure pain and the cold pressor task, but not on heat or electrical pain, with the magnitude of effect greater for 65 mg compared with 15 mg for the cold pressor but similar for the pressure pain test, and smaller than that observed with pregabalin, in healthy participants (van Amerongen et al., 2019). Given that this trial was executed in parallel with a clinical trial of darigabat in patients with pain the PainCart healthy participant trial was not used as decision-making to proceed into patients with pain. As such, the learnings from this trial related to RO and the quantitative comparison between the 2 doses of darigabat tested in the trial compared to pregabalin was not considered in the design of the chronic low back pain trial which is discussed in **Chapter 3**.

Modulation of biomarkers do not necessarily indicate what disorder the NME is most likely to be effective in, or which dose will be required to achieve clinically meaningful benefit. However, biomarkers may be used to synthesise knowledge of the efficacy, tolerability, the pharmacology, and the potential therapeutic role of a novel drug in research. The biomarkers utilised in the darigabat research and development program confirmed target engagement and pharmacology, that the nonclinical profile translated to the clinical profile, produced data suggestive of anxiolytic and analgesic potential, and overall demonstrated a differentiated profile compared to classical BZDs, with high RO achievable due to minimal potentiation of the α1-containing $GABA_A$ receptors. This catalogue of data makes darigabat an ideal tool to further study the potential benefits of this selective mechanism in patients with pain, anxiety disorder, and seizures.

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3 EFFICACY

3.1 Introduction

One of the biggest challenges in drug development for an unprecedented mechanism is understanding the relationship between target tissue exposure and efficacy. Whilst biomarkers may provide confirmation that a molecule delivers exposure at the target site of action and elicits the desired target modulation, they are often complementary to rather than replacements for the studies that are designed to measure efficacy in the target patient population. Furthermore, predicting the magnitude of response (i.e., degree of efficacy) in humans using biomarker data is somewhat elusive. As such, lack of efficacy is a key cause of attrition in research and development, accounting for in excess of 30% of failures for CNS drugs (Kaitin and DiMasi, 2011).

Darigabat was studied in nonclinical models of pain, anxiety, and epilepsy to provide confidence in the association between selective GABAergic modulation and efficacy prior to the conduct of clinical trials in each of these patient populations. The contribution of the author to these works is outlined in **Table 1-1** in **Chapter 1**.

3.2 Nonclinical efficacy studies

Molecular genetic studies with α-subunit knockout and point-mutated mice approximately 20 years ago have underpinned our understanding of $GABA_A$ receptor and benzodiazepine (BZD) pharmacology (Rudolph et al., 1999; Crestani et al., 2000; Low et al., 2000; McKernan et al., 2000; Rudolph and Mohler, 2006; Fradley et al., 2007; Knabl et al., 2008 and 2009). This fundamental work combined knocking out specific populations of $GABA_A$ receptors with *in vivo* behavioural studies of sedation, addiction, anxiety, pain, epilepsy, and cognition and has developed our understanding of the contribution of each receptor subpopulation with pharmacological effects. These same behavioural models have then been utilised to confirm the *in vivo* pharmacology of subtype-selective tool compounds and is the basis of this collective data that delineates subtypes into α1 = sedation, α1/2 = convulsant; α2/3 = anxiety, α2/3/5 = pain, α5 = cognition (Crestani et al., 2000; Collinson et al., 2002; Crestani et al., 2002; Atack et al., 2005; Dias et al., 2005; Dawson et al., 2006; Maubach, 2006; Atack et al., 2009; Martin et al., 2009). Although this is undoubtedly an oversimplification, it is the basis for the hypothesis that compounds with preferential α1 agonist activity should be sedatives,

activity at α2/3 should be anxiolytic and α5-selective inverse agonists should improve cognitive function.

The darigabat program has utilised nonclinical models of pain, anxiety, and seizures to increase confidence in rationale that the *in vitro* profile was aligned with the hypothesised safety and efficacy profile of a selective PAM (Nickolls et al., 2011; Duveau et al., 2019; Owen et al., 2019).

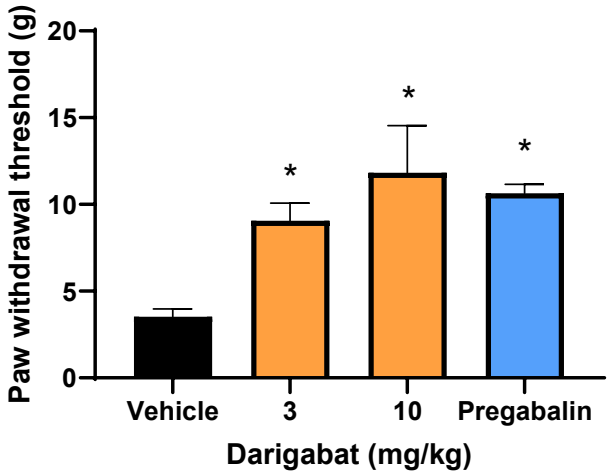
3.2.1 Nonclinical pain models

An inflammatory pain model and a range of neuropathic pain models in rodents were selected to examine the analgesic profile of subtype-selective PAMs early in the research program. Some of the evidence of analgesic efficacy via modulation of α2 and α3 $GABA_A$ receptors using both diazepam and L-838417 was elucidated in the chronic constriction injury (CCI) model of neuropathic pain and hence this model was selected for further study for the darigabat program. Another subtype-selective PAM, NS11394, was investigated in the spared nerve and CCI neuropathic pain models and in the complete Freund's adjuvant (CFA) inflammatory model (Munro et al., 2008), adding further weight to the analgesic potential of this mechanism of action (MOA) in nonclinical models of both neuropathic and inflammatory pain. As such, during the prototype discovery phase of the program, tool compounds L-838417 and TPA023 (the latter of which has lower *in vitro* functional activity at α2/3 compared to L-838417) were investigated in rodent models of inflammatory and neuropathic pain following oral administration (Nickolls et al., 2011). Briefly, inflammation is established by intraplantar injection of CFA which results in hyperalgesia which can be quantitatively measured using paw withdrawal latency to a heat stimulus and can be reversed by ibuprofen. The neuropathic pain models involve transection, ligation, or constriction of nerves innervating the hind paws which results in a neuropathic pain state including symptoms such as allodynia which can be measured by assessing paw withdrawal thresholds to von Frey filaments of differing weights and can be reversed by pregabalin. In brief, von Frey filaments ranging from 0.4 to 15.0 g were applied to the plantar surface of the ipsilateral hind paw, starting with a 2.0 g filament. Filaments were then presented in an ascending or descending pattern, depending on the animal's responses. Each von Frey filament was applied until a

withdrawal response was obtained, up to a maximum of six seconds. This was repeated on the contralateral hind paw. Animals were considered to be allodynic if the ipsilateral 50% paw withdrawal threshold (50% PWT) value calculated using this paradigm was 4.0 g or less. At > 60% $GABA_A$ receptor occupancy (RO) L-838417 dose-dependently demonstrated efficacy in the inflammatory CFA model whereas TPA023 did not, even at ~ 98% RO (Figure 2 in Nickolls et al., 2011). There were also mixed results in 3 models of neuropathic pain, with neither compound showing efficacy in the tibial nerve transection model even at near 100% RO, but both compounds demonstrated some efficacy in the CCI and spared nerve ligation models, with TPA023 requiring higher RO than L-838417 to exert analgesia (Figures 3-5 in Nickolls et al., 2011). There is a not a conclusive explanation for the different effects of $GABA_A$ receptor PAMs across the neuropathic pain models, as differences between the models are not well understood. Furthermore, it is likely that L-838417 is not completely devoid of α1-subunit activity and sedation can influence paw withdrawal latency in the evoked endpoints used in nonclinical models (Nickolls et al., 2011; Nickolls et al., 2018). Nevertheless, data with both compounds highlight the importance of RO, with even the higher intrinsic efficacy compound L-838417 requiring 60% of receptors to be occupied to exhibit significant efficacy.

As reported in Owen et al., 2019, orally administered darigabat (Compound 34) was efficacious in the CCI model of neuropathic pain at 3 and 10 mg/kg, corresponding to approximately 70 and 90% RO, respectively (**Figure 3-1**). Lower doses were not tested so it is not possible to conclude that a compound with intermediate α2/3 functional efficacy (i.e., L-838417 > darigabat > TPA023) requires higher RO than a compound with higher functional efficacy and vice versa. However, the totality of the nonclinical data is suggestive of the analgesic potential of subtype-selective compounds like darigabat.

Figure 3-1 The effect of darigabat on chronic constriction injury-induced allodynia in the rat

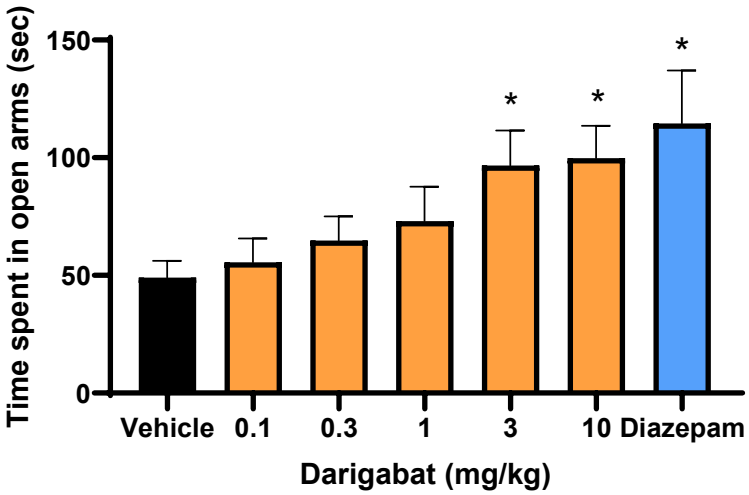


Allodynia was assessed by recording the pressure at which the paw was withdrawn in response to graded stimuli (using von Frey filaments). Paw withdrawal threshold was measured at 2-hours post oral administration. 30 mg/kg pregabalin was used as the positive control. Data are means ± standard error of the mean, n=8 per group. * = P<0.05, two-way ANOVA comparing all doses to vehicle followed by Bonferroni post-tests. (Owen et al., 2019).

3.2.2 Nonclinical anxiety model

The elevated plus maze in rodents is a behavioural model widely used to assess the anxiolytic effects of pharmacological agents. Briefly, rodents are placed at the junction of the four arms of a maze, facing an open arm, and entries/duration of time spent in each arm are recorded. An increase in time spent in the open arm reflects anti-anxiety behaviour, an outcome that is observed following treatment with BZDs and subtype-selective PAMs including L-838417 (at < 50% RO) and TPA023 (at ≥ 70% RO) (McKernan et al., 2000; Atack et al., 2011). For the darigabat study, comparisons were made between orally administered vehicle, diazepam (3 mg/kg) and darigabat (0.1, 0.3, 3 and 10 mg/kg; Owen et al., 2019). Darigabat (3 and 10 mg/kg) produced robust anxiolytic-like effects similar in magnitude to that of diazepam (**Figure 3-2**). The plasma levels of darigabat in this study at the given doses were lower than anticipated and achieved approximately 50% RO at the highest dose.

Figure 3-2 The effect of darigabat on anxiolysis induced by the elevated-plus maze in mice



Anxiolytic-like activity was assessed by recording the time spent in the open arms of the elevated-plus maze at 1-hour post oral administration. 3 mg/kg diazepam was used as the positive control. Data are means ± standard error of the mean, n=10 per group. * = P<0.05, one-way ANOVA with Dunnett's post-hoc test (vehicle versus darigabat) and unpaired t-test (vehicle versus diazepam). (Owen et al., 2019).

3.2.3 Nonclinical seizure models

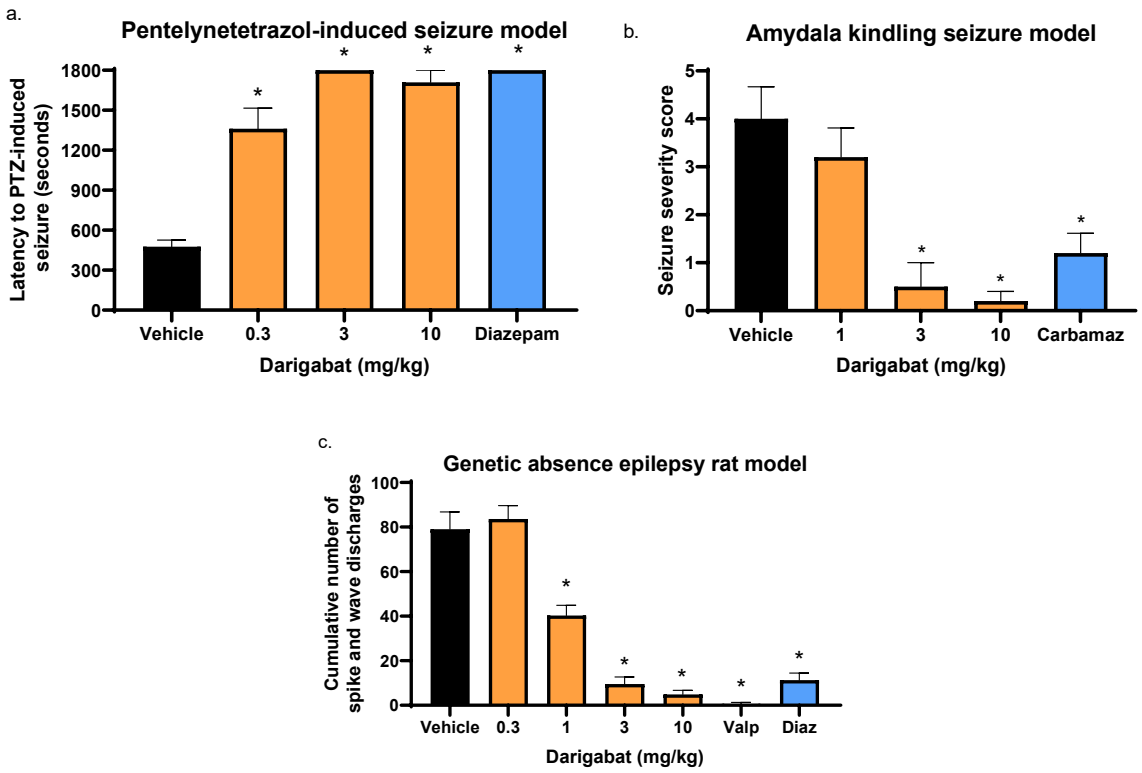
Nonclinical models of epilepsy have facilitated the identification of valuable drugs for the symptomatic treatment of seizures. One of the most widely used screening tools in anticonvulsant drug research has been the pentylenetetrazol (PTZ) model which has been used for > 70 years. PTZ, a $GABA_A$ receptor antagonist, is administered and latency to convulsion or dose required to induce a convulsion is used to assess the anti- or pro-convulsant activity of compounds. The test is reported to be predictive of efficacy in nonconvulsive (absence or myoclonic) seizures (Krall et al., 1978). However, more recently several antiepileptic drugs (AEDs) that are efficacious in patients with nonconvulsive seizures failed in PTZ, highlighting the need for other models of nonconvulsive seizures. Nevertheless, the PTZ model has been used to understand the pharmacology of BZDs in molecular knock-out mice and to examine the potential efficacy of subtype-selective $GABA_A$ receptor PAMs, including L-838417 and TPA023. In the

transgenic mice, no single α subtype was found to be responsible for the anticonvulsant effects of diazepam in the PTZ model, although neither the α3 nor α5 subtypes appeared to confer anticonvulsant activity, with both α1 and α2 playing a role (Fradley et al., 2007). Data with α1-sparing PAMs underscored the potential importance of the α2-subunits in the anticonvulsant efficacy of BZDs. L-838417 had anticonvulsant activity at doses that occupied less than 50% of BZD binding sites, whilst TPA023 at approximately 50% RO gave 50% protection from PTZ-induced seizures (Atack et al., 2006). Darigabat administered orally to mice significantly increased seizure latency in the PTZ model at all doses tested (0.3, 3, and 10 mg/kg) which spanned RO ranges from ~48-95% (**Figure 3-3a**; Owen et al., 2019; Bialer et al., 2020).

Amygdala kindling in which temporal lobe epilepsies are induced by repeated electrical stimulation of the amygdala, is a well-characterized model for focal seizures and activity in this model has been shown to be predictive of efficacy in focal to bilateral tonic-clonic seizures in the clinical setting (Loscher, 2011). The kindling model accurately predicts the clinical utility of all the AEDs currently employed in the treatment of focal seizures (Klitgaard et al., 2008). Moreover, the kindled rat is the one model that accurately predicted the clinical utility of levetiracetam, one of the mostly widely used approved AEDs, highlighting the importance of using a battery of models during initial screening to avoid disregarding a potential new therapy. No data is reported with L-838417 or TPA023 in the amygdala kindling model but darigabat when administered orally at 3 mg/kg and 10 mg/kg displayed potent activity in fully kindled rats, providing protection against focal to bilateral tonic-clonic seizures and significantly inhibited or reduced seizure severity (**Figure 3-3b**; Owen et al., 2019).

Whilst amygdala kindling models focal onset seizures in humans, the genetic absence epilepsy rat from Strasbourg (GAERS) models generalised (absence) seizures and has been shown to be predictive of an AED's ability to both suppress and aggravate this form of clinical epilepsy (Duveau et al., 2018). The GAERS model uses a selectively inbred strain of Wistar rats exhibiting spontaneous spike-and-wave discharges (SWDs) reminiscent of clinically observed absence seizures. These rats present with behavioural, electrophysiological, and pharmacological features of absence seizures and as such is a

Figure 3-3 The effect of darigabat in rodent models of seizures



Abbreviations: Carbamaz = carbamazepine; Diaz = diazepam; PTZ = pentylenetetrazol; SWD = spike-and-wave discharges; Valp = valproate.

Each graph shows peak effect after oral administration of darigabat. Data are means ± standard error of the mean. (a) Latency to first clonic response in pentylenetetrazol-induced seizures was assessed in mice 1-hour post-administration, n=10 per group. 1.5 mg/kg diazepam administered orally used as the positive control. * = P<0.05, repeated measures ANOVA with Dunnett's post-hoc test (compared to vehicle treatment group). (b) Amygdala kindling-induced seizure severity score was assessed in rats 1-hour post administration, n=10 per group. 30 mg/kg carbamazepine administered intraperitoneally was used as the positive control. * = P<0.05, repeated measures ANOVA with Dunnett's post-hoc test (compared to vehicle treatment group). (c) Spike-and-wave discharges in rats with genetic absence epilepsy was assessed during the first hour post-treatment, n=8 per group. 500 mg/kg valproate and 3 mg/kg diazepam administered orally used as the positive controls. * = P<0.05, repeated measures ANOVA (compared to vehicle treatment group). (Duveau et al., 2018; Owen et al., 2019; Bialer et al., 2020).

favourable model to investigate the efficacy of novel AEDs with varied mechanisms of actions. Darigabat (0.3, 1, 3, 10 mg/kg administered orally) demonstrated robust dose-dependent efficacy, reducing the expression of SWDs, including full suppression at the highest doses by 30 minutes after administration, with the minimal effective dose (0.3 mg/kg) achieving approximately 57% RO (**Figure 3-3c**). This data confirmed broad spectrum of efficacy of darigabat in different seizure subtypes.

In summary, the nonclinical data with darigabat demonstrates efficacy in models of pain, anxiety, and epilepsy, and that at least approximately 50% of the $GABA_A$ receptors need to be occupied by darigabat to derive benefit in rodent species.

3.3 Clinical efficacy studies

Darigabat has been investigated in three completed Phase 2 clinical trials in patients with chronic low back pain, anxiety, and epilepsy (**Table 3-1**). The indications were selected based on the strength of evidence primarily gathered in nonclinical studies, plus on the continued clinical unmet need, precedented regulatory path, and commercial potential. Although positive data with a subtype selective $GABA_A$ receptor PAM has been reported previously in patients with anxiety (Atack et al., 2006), to our knowledge these were the first trials investigating this MOA in randomised, controlled trials in pain and epilepsy patients.

At the time of conducting these clinical trials, an arbitrary dosing cap was imposed which resulted in multiple doses in clinical trials at that time being limited to a maximum of 7.5 mg BID (approximately 60% RO; **Chapter 4.2.1**).

Table 3-1 Summary of completed Phase 2 clinical trials in patients

Protocol No./ NCT ID (Status)	Trial Design	Key Objectives	Treatment/Doses	Total no. of patients (received darigabat)	Key efficacy findings
B7431005/ NCT2564029 (Completed February 2017)	Randomised, Phase 2, double blind, placebo- and active-controlled, cross-over, multi-centre, single oral dose trial in patients with photosensitive epilepsy.	Assess ability of darigabat to reduce the photosensitivity range in patients with documented photosensitive epilepsy.	Single oral doses of 17.5 and 52.5 mg darigabat, 2 mg lorazepam and placebo.	7 (7)	Darigabat at both doses and lorazepam 2 mg (positive control) significantly reduced the photosensitivity range ($P < 0.05$, fixed effect model one-sided test), and it was completely suppressed in 6/7 patients.
B7431006/ NCT02262754 (Stopped August 2015)	Randomised, Phase 2, double-blind, placebo- and active-controlled, parallel-group, multi-centre, multiple oral dose trial in patients with chronic low back pain.	Assess the analgesic efficacy of darigabat in patients with chronic low back pain.	Oraly administered 2.5 mg BID for 1 week followed by 7.5 mg BID of darigabat for 3 weeks, naproxen 500 mg, placebo.	222 (74)	The trial was stopped early following an interim analysis which showed that darigabat showed no analgesic efficacy. Naproxen (positive control) reduced pain in line with expectations.
B7431007/ NCT02310568 (Stopped October 2015)	Randomised, Phase 2, double-blind, placebo-controlled, multi-centre, sequential parallel-group, multiple oral dose trial in patients with generalised anxiety disorder.	Assess anxiolytic efficacy of darigabat in patients with inadequate response to generalised anxiety disorder monotherapy.	Orally administered placebo, 2.5 mg BID darigabat for 4 weeks and 2.5 mg BID for 1 week followed by 7.5 mg BID darigabat for 3 weeks.	90 (72)	The trial was stopped early for business prioritisation reasons. Darigabat did not show any anxiolytic activity.

Abbreviations: AE = adverse event; BID = twice daily dosing; NCT ID= ClinicalTrials.gov Identifier; No. = number.

3.3.1 Phase 2 trial in patients with chronic low back pain

$GABA_A$ receptors as targets for pain gained support predominantly from nonclinical studies, including in point mutant diazepam-insensitive mice which have shown that positive modulation of GABA action at α2- and α3-containing $GABA_A$ receptors in the spinal cord results in pain relief (Knabl et al., 2008). These data have further been confirmed nonclinically using compounds with equivalent binding affinity for the different α subunits, but subtype-selective functional activity (Munro et al., 2008; Nickolls et al., 2011). Whilst BZDs are not indicated for pain control, they are frequently taken by pain patients and prescriptions of BZDs to treat pain is rising in the US (Agarwal and Landon, 2019). This is particular concern in pain patients due to the potential concurrent use with opioids, with the National Institute on Drug Abuse indicating in 2019 that approximately 30% of opioid-induced overdose deaths involving the use of a BZD.

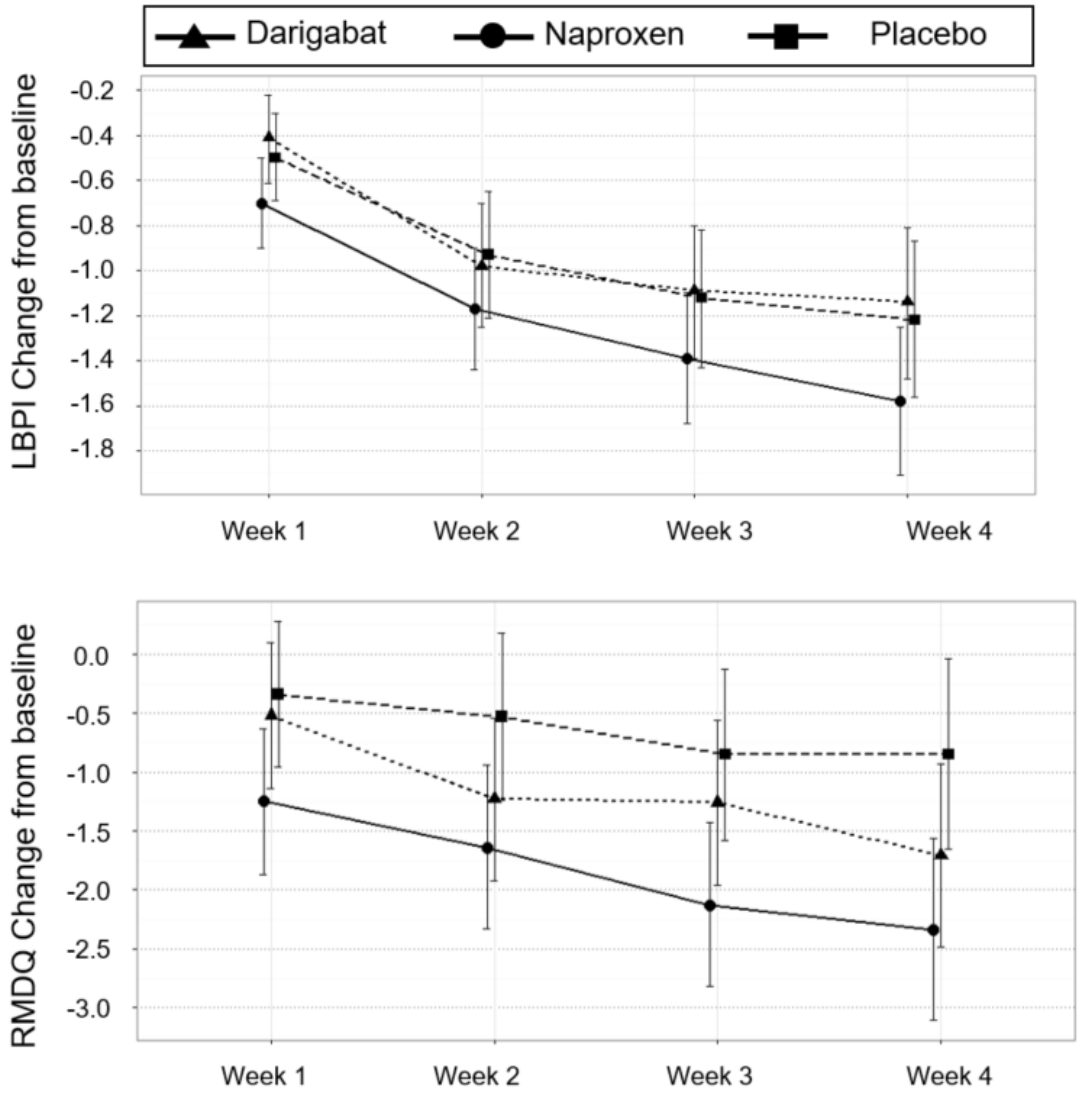
Chronic low back pain (CLBP) was selected as the primary pain indication for darigabat following research to identify both positive and negative clinical trial data in patients with pain using BZDs, focusing on large pain populations with continued unmet needs (unpublished). That analysis found little evidence for utility in neuropathic pain conditions such as post-herpetic neuralgia, diabetic peripheral neuropathy, or trigeminal neuralgia (Brotz et al., 2010). No specific rationale was identified for BZD use in osteoarthritis. However, three clinical studies with the BZD tetrazepam in the 1990s demonstrated efficacy in this population (Arbus et al., 1990; Salzmann et al., 1992; Salzmann et al., 1993), highlighting the potential opportunity to offer symptomatic relief through both GABA-mediated analgesia and muscle-relaxant activities. The trial was designed and initiated prior to data being available from the PainCart trial with darigabat (van Amerongen et al., 2019), and when there was a self-imposed dose-cap in place, limiting the highest dose in the trial to 7.5 mg BID (~60% RO at $C_{max;}$ see **Chapter 4.2.1**).

The randomised, double-blind, placebo- and active-controlled proof-of-concept (POC) trial to investigate the effect of darigabat in patients with CLBP was initiated in November 2014 (Gurrell et al., 2018). The parallel treatment group trial consisted of a 1 week single-blind placebo run-in phase, followed by 4 weeks double-blind treatment consisting of

either darigabat 2.5 mg BID for one week followed by darigabat 7.5 mg BID for 3 weeks, or naproxen 500 mg BID for 4 weeks or placebo BID for 4 weeks. The trial planned to randomise 300 patients in a 1:1:1 ratio darigabat:naproxen:placebo. Patients aged 18-75 years were eligible for the trial if they had a duration of CLBP for ≥ 3 months requiring regular use of analgesic medication (paracetamol alone was not permitted as the only analgesic medication) and if at screening they self-reported their average pain score of ≥4 and ≤9 for low back pain intensity (LBPI) on a Numeric Rating Scale (NRS). There was active exclusion of participants exhibiting CLBP with a neuropathic component, and those meeting criteria related to clinically significant anxiety and/or depression. Patients were only randomised if they had an average daily NRS of LBPI during the patient-blinded placebo run-in of ≥ 4 and ≤9, and demonstrated the required compliance related to both tablet administration and completion of their daily pain diaries.

The primary endpoint was the NRS of LBPI after 4 weeks of active treatment, secondary endpoints included the Roland Morris Disability Questionnaire (RMDQ) and the Hopkins Verbal Learning Task-Revised (HVLT-R). The trial had predefined decision rules based on likelihood that darigabat was better or worse than placebo (considered to be a 0.8 reduction in LBPI NRS compared to placebo based on unpublished internal analysis of previous CLBP trial data). An interim analysis performed by a team separate from the blinded trial team was planned when 50% of patients had completed the primary endpoint. A total of 222 participants were randomised to 1 of 3 active treatment groups. The mean darigabat 4-week response on the LBPI was 0.16 units higher (worse) than placebo (90% confidence interval -0.28, 0.60), and -0.26 (-0.70, 0.18) for naproxen, indicating that whilst naproxen was efficacious, darigabat was not differentiated from placebo and the trial was stopped at the interim analysis for futility (**Figure 3-4**). There was however evidence of an improvement over placebo with darigabat in the RMDQ, and a reduction in the HVLT-R delayed recall test score, both indicators that whilst analgesia was not detected in this trial as measured by the primary endpoint, there was some evidence of pharmacology exerted by darigabat that was maintained for the 4-week dosing period of the trial.

Figure 3-4 Least square mean profile plot of weekly low back pain intensity and Roland Morris Disability Questionnaire scores



Abbreviations: LBPI = low back pain intensity; RMDQ = Roland Morris Disability Questionnaire.

The effect of darigabat administered for 4 weeks on primary (LBPI) and secondary (RMDQ) endpoints in patients with chronic low back pain. Least square mean profile plot of week LBPI and RMDQ scores from the mixed-model repeated measures (MMRM) analysis. The MMRM model includes treatment, week, baseline, the week x treatment interaction, and the baseline x week interaction as fixed effects and week repeated within subject as a repeated effect. Baseline is included as a covariate. Adapted from Gurrell et al, 2018.

There are several factors that could have contributed to the disappointing lack of analgesic activity of darigabat in this trial. These include the fundamental role of $GABA_A$ receptors in analgesia, the pharmacology of darigabat (i.e., level of functional activity), a failure in the translation of efficacy from nonclinical species to the clinic, the specific pain subpopulation selected for the trial and attributes of the clinical trial design such a dose selection.

In 1965, the gate control theory of pain first postulated the role of GABAergic inhibitory tone in pain perception (Melzack and Wall, 1965). Subsequent to this, anatomical and functional evidence demonstrated loss of GABAergic tone contributed to spinal hyperalgesia after injury (Sivilotti and Woolf, 1994; Enna and McCarson, 2006). Renewed interest in $GABA_A$ receptors as targets for pain then came from studies using point-mutated BZD-insensitive mice. These showed that potentiation of spinal α2/3-containing $GABA_A$ receptors produced analgesia (Knabl et al., 2008 and 2009). Spinal expression patterns of $GABA_A$ receptor subunits are largely conserved between rodents and humans (Waldvogel et al., 1990 and 2010) and differences in the functional roles may exist, although spinal $GABA_A$ receptors are important for analgesia in both rodents (Knabl et al., 2009) and humans (Goodchild and Nobel, 1987; Serrao et al., 1992). These findings suggest that fundamental differences in the functional roles of $GABA_A$ receptors are less likely to be the cause of the negative result in this trial.

Although there is little published evidence to suggest that BZDs are effective in CLBP with a neuropathic component (Chou et al., 2007; Brotz et al., 2010; Cohen, 2010), a nonclinical neuropathic pain model was selected to determine the analgesic potential of darigabat. The selection was made based on the efficacy of BZDs in the neuropathic pain models, including those in the point-mutated BZD-insensitive mice (Knabl et al., 2008 and 2009), and given the lack of availability of a rodent model of CLBP. In rodents, potentiation of α2/3 $GABA_A$ receptors alleviates inflammatory and neuropathic hyperalgesia but does not interfere with responses to acute nociceptive stimuli (Knabl et al., 2008) and low back pain is considered a nociceptive pain condition in most patients (Beith et al., 2011). Moreover, patients with CLBP with a neuropathic component were actively excluded from this trial. The discrepancy between the effectiveness of BZDs in nonclinical neuropathic

models and patients experiencing neuropathic pain could be due in part to the inability to reach high enough RO in humans given the sensitivity to sedation. The potential failure of translation may also not only relate to the model and pain state, but also to the differences in endpoints within studies nonclinically versus clinically. Rodent models of pain in general either involve induction of hyperalgesia by damage of peripheral nerves (e.g., CCI) or inflammation (e.g., local injection of CFA), and changes in pain thresholds are measured by withdrawal behaviours from thermal or mechanical stimuli. In contrast to nonclinical models, pain is measured in clinical trials largely through subjective pain ratings scales, such as the NRS used as the primary end point in the trial with darigabat. Methods more akin to the animal model determination of pain thresholds are available in the form of quantitative sensory testing (Rolke et al., 2006) and several studies have found clobazam has an effect in hyperalgesia models, but not on pain thresholds (Vuilleumier et al., 2013; Besson et al., 2015; Schliessbach et al., 2017). These data suggest that beneficial effects of $GABA_A$ receptor PAMs may be limited to patients who have hyperalgesia, which was not assessed in this trial.

Whilst there is robust evidence that subtype selective PAMs are analgesic nonclinically, evidence of the clinical analgesic potential of darigabat was observed in the PainCart in healthy participants (van Amerongen et al., 2019). In this single dose trial 15 mg (achieving ~ 60% RO) darigabat increased pain tolerance thresholds to pressure pain, and 65 mg (~80% RO) increased both pressure pain and pain tolerance threshold for cold pressor and a trend to do so in electrical stimulation. In general, the effect size was pregabalin > darigabat 65 mg > darigabat 15 mg, and suggests in this multimodal model, albeit in healthy participants, that the effect of darigabat is modest, particularly at the lower 15 mg dose which results in approximately equivalent concentrations to the 7.5 mg BID dose selected for the CLBP trial. The determination of pain thresholds evoked by a range of stimuli in PainCart are more akin to the endpoints used in nonclinical models than the subjective rating scales used in the CLBP trial. In common with nonclinical models of pain, experimental medicine models of pain can be advantageous for homogenously characterising potential new analgesics as they do not have the same confounders – the investigator can control the experimentally induced pain (including the nature,

localisation, intensity, frequency, and stimulus duration). It does not mimic heterogenous pain experience in chronic pain patients and experimental models do not have the same psychological effects of someone experiencing chronic pain. When treating clinical pain analgesic effects are difficult to evaluate due to several factors other than pain intensity, such as psychological, cognitive, and social impacts of the illness. Hence changes in these factors will invariably interfere with pain intensity and quality and impact the evaluation of a novel analgesic. However, the data is suggestive of some antihyperalgesic potential of darigabat, particularly at the higher dose which achieved > 80% RO.

Although the PainCart data was not available at the time of initiating the CLBP trial with darigabat, data with tetrazepam and clobazam in clinical trials, including 3 positive trials in CLBP with the former, provided strong mechanistic rationale for $GABA_A$ receptor potentiation in analgesia (Arbus et al., 1990; Salzmann et al., 1992; Salzmann et al., 1993; Schliessbach et al., 2017). Both are nonselective BZD PAMs with high functional efficacy at the α1/2/3/5-containing receptors. In contrast, darigabat has comparatively much lower functional activity at α2/3/5-containing receptors (Nickolls et al., 2018). There is evidence that PAMs with lower functional efficacy need to occupy a greater proportion of the receptors to produce the same behavioural effect as a PAM with higher functional efficacy in nonclinical models of anxiety (Atack, 2010), and pain as exemplified by TPA023 which was effective in only 2 of 4 pain models (Nickolls et al., 2011). This suggests that there is potentially a threshold under which PAMs with lower functional efficacy than BZDs are not effective. Furthermore, studies in mice indicate that the RO for antihyperalgesic effects is much higher than those for sedation (Ralvenius et al., 2015). Thus, it may be that significant antihyperalgesia is only obtained in humans at very high RO.

Nevertheless, there are lessons to be learnt from this trial. Both the nonclinical and, retrospectively, the PainCart data evidenced that the higher the RO the better the opportunity to observe efficacy in pain. If the PainCart data had been available prior to the initiation of the CLBP trial perhaps the latter trial would not have been initiated, or at least not initiated until further safety data was available to support chronic administration of doses to achieve higher than the 60% RO achieved with 7.5 mg BID. As such it remains

unclear whether selectively targeting α2/3/5-subunits of $GABA_A$ receptors has analgesic potential in patients with pain.

3.3.2 Phase 2 trial in patients with photoepilepsy

Clinically, the use of drugs to modulate GABA in epilepsy is well-precedented, including the use the barbiturate phenobarbital more than 100 years ago, BZD use from the 1960s onwards, and more recently with drugs that increase the availability of GABA at the synapse by either preventing its breakdown or re-uptake. Nonclinical data has implicated both α1- and α2-containing $GABA_A$ receptors for the anticonvulsant activity of BZDs (Rudolph et al., 1999; Fradley et al., 2007; Duveau et al., 2019). Despite the strong nonclinical evidence that modulation of α2-containing $GABA_A$ receptors with darigabat would result in anticonvulsant activity, a cautious approach was taken in moving forward into epilepsy patients for multiple reasons.

First, there had been no trial to confirm that selective potentiation of GABA via α2-containing subunits would have the horsepower to reduce or prevent seizures in patients with epilepsy. Epilepsy is a serious condition and is associated with a risk of sudden-unexpected death in epilepsy (SUDEP), a risk best mitigated by controlling seizures and, as such, exposing large numbers of patients to both a novel drug with limited/no clinical rationale and to placebo poses ethical issues. Second, there has been some disconnect between the *in vitro* functional profile and the pharmacology expressed in the clinic with some subtype-selective $GABA_A$ receptor PAMs which have minimal *in vitro* α1 activity and have resulted in dose-limiting sedation/drowsiness such as with MK-0343, TPA023, and TPA023B (Atack, 2009). Therefore, a disconnect between the positive α2-related anticonvulsant nonclinical data translating to a positive clinical outcome could not be ruled out. Third, as darigabat binds to but has minimal activity at α1-containing receptors, hypothetically if a patient with epilepsy needed “rescuing” from status epilepticus with a BZD, there is a theoretical risk that the BZD may not be as effective with co-administration of darigabat as it may preferentially “block” those receptors due to the higher binding affinity of darigabat versus BZDs. For these reasons, there was a desire to confirm confidence in rationale in a small inpatient trial prior to embarking on a traditional outpatient trial in large numbers of epilepsy patients on adjunctive AEDs. Several different

trial options were available which had the potential to increase confidence in rationale that α2 potentiation at high RO in patients with epilepsy would result in anticonvulsant activity.

A pre-surgical model has previously been used to uncover potential efficacy in reducing seizures (Pledger and Kramer, 1991). In this model, patients with refractory seizures who are candidates for surgical treatment of their epilepsy are evaluated inpatient following withdrawal of their AEDs. Following washout of their existing AED treatment and confirmation they meet eligibility criteria related to seizure type and frequency they are randomised to receive active treatment or placebo/active comparator. The efficacy assessments are different to traditional epilepsy trials and include time to discontinue from randomised treatment (for example, have their fourth seizure, or number of seizures has reached pre-randomisation levels), the proportion of participants completing the trial, average daily seizure frequency, and proportion of participants that are seizure-free. The advantages of this pre-surgical model include that efficacy is evaluated in a short time-period with relatively few participants, the novel drug is evaluated as a monotherapy and is not constrained to add-on therapy as traditional trials are, and that there are fewer ethical risks with use of placebo due to the short-term controlled inpatient environment. The disadvantages of this trial design include whether the endpoints in this short-term trial are predictive of sustained efficacy during long-term treatment, and the fact that these patients are likely super-refractory to AED treatment, although now approved AEDs such as felbamate (Bourgeois et al., 1993; Devinsky et al., 1995), gabapentin (Bergey et al., 1997), and oxcarbazepine (Schachter et al., 1999) would suggest that despite this, treatment effects may be observed. This trial design appears to have not been utilised (or at least published on) for nearly 20 years, potentially due to the difficulty in recruiting patients, particularly given that the number of AEDs and implantable electrical stimulators now available as treatment options before reaching the conclusion that surgery may be the most appropriate treatment approach. The perceived difficulty in recruiting patients to participate in a trial of this type meant that it was not further pursued, despite its potential to provide proof-of-principle (POP) of anticonvulsant activity of darigabat.

Another subset of patients with epilepsy that have participated in trials used to determine anticonvulsant activity early in drug development are those with photosensitive epilepsy. Photosensitive epilepsy is a reflex form of epilepsy, in which photically-induced generalised epileptiform responses on EEG, called photoparoxysmal responses (PPR) can be evaluated prior to and after drug administration (Kasteleijn-Nolst Trenite et al., 1996). Utilisation of photic-evoked epileptiform discharges, which can be produced on demand, circumvents the fact that epileptic seizures occur in a random, unpredictable pattern, which would eliminate the possibility of assessing drug response with a single dose. This model has been used successfully to evaluate potential antiseizure effects of new drugs in small groups of patients (French et al., 2014), including most recently with the newly approved antiepileptic drug cenobamate in just 3 participants per dose level tested (Kasteleijn-Nolst Trenite et al., 2019), and at relatively low cost. Indeed, a statistical analysis of previous data in the model (French et al., 2014) with commonly used second generation AED levetiracetam had shown that 6 participants completing a cross-over design with darigabat would be appropriate to detect an effect of drug with 80% probability. Additionally, single dose photosensitive POP trials with drugs with multiple mechanisms of actions have been shown to be useful as early and informative indicators in AED discovery and development, particularly in aiding dose selection in Phase 2 and 3 epilepsy trials (Yuen and Sims, 2014). A further advantage of the model is that it is conducted inpatient under the care of an epileptologist and is designed in such a way that although the hallmark of epileptic activity is observed on EEG, seizures are not precipitated to reduce the risk to the patient (who remain on their current AED therapies). Some of the draw backs of this trial design are that the endpoint that is measured, the standardised photosensitivity range, is not a registrable clinical outcome assessment for epilepsy clinical trials. Also, given that the studies are a single dose administration it does not provide information on the maintenance of efficacy, which is a particular issue associated with BZDs. Additionally, as there is a “floor effect” at which the participant is no longer photosensitive, data from this trial cannot be used to quantitatively predict the level of efficacy that may be observed in a traditional clinical trial using the registration endpoint. Nevertheless, the trial validity and predictivity as exemplified by numerous

AEDs of different mechanisms and ability to determine the potential utility of darigabat at relatively low cost in epilepsy patients with quick data readout proved an attractive proposition. In fact, the data from this trial proved to be pivotal to the continued investment of the whole program, both at Pfizer initially and in attracting new buyers for the Pfizer neuroscience portfolio.

Most prior studies in the photosensitivity model have been single blind, inpatient studies, where the treatment day would immediately follow the placebo day and lacked the ability to study more than one active treatment within each patient. This trial used the double-blind, cross-over design approach demonstrated by French et al., 2014 to examine 2 doses of darigabat and permitted the inclusion of the active control lorazepam to assess trial sensitivity, a comparator relevant to the understanding of the novel mechanism under investigation (Gurrell et al., 2019). Key design aspects also included that photosensitivity was measured at 0 (pre-dose), 1, 2, 4- and 6-hours post-dose to cover the period at which peak darigabat exposure and pharmacodynamic responses had been observed (Nickolls et al., 2018), but also so that the trial would not require an overnight stay at the trial site. After confirmation that patients met all the eligibility criteria at screening, they were randomised to one of 4 treatment sequences based on a single Latin square design. Participants attended the investigator site for the active treatment periods with a minimum of 1 week and a maximum of 3 weeks treatment washout between active treatment periods. A minimum of 1 week would allow, based on the half-lives of the single doses of treatments, for sufficient time to wash-out drug and effects on photosensitivity ranges.

The doses of darigabat were selected to cover both the lower RO following administration of a single 17.5 mg dose, which at that time was permissible to take forward into a multiple dose clinical trial, and a high 52.5 mg single dose to achieve approximately 80% RO, the latter of which representing a ‘no-regrets’ dose at which negative data would indicate that α2/3/5-subtype-selective PAMs likely did not have a utility in the treatment of seizures.

The trial also utilised prespecified decision criteria which were developed using data from the previous trial with levetiracetam (French et al., 2014) and were used to identify quantitively what level of change in photosensitivity range would constitute a desired effect level in at least 6 participants.

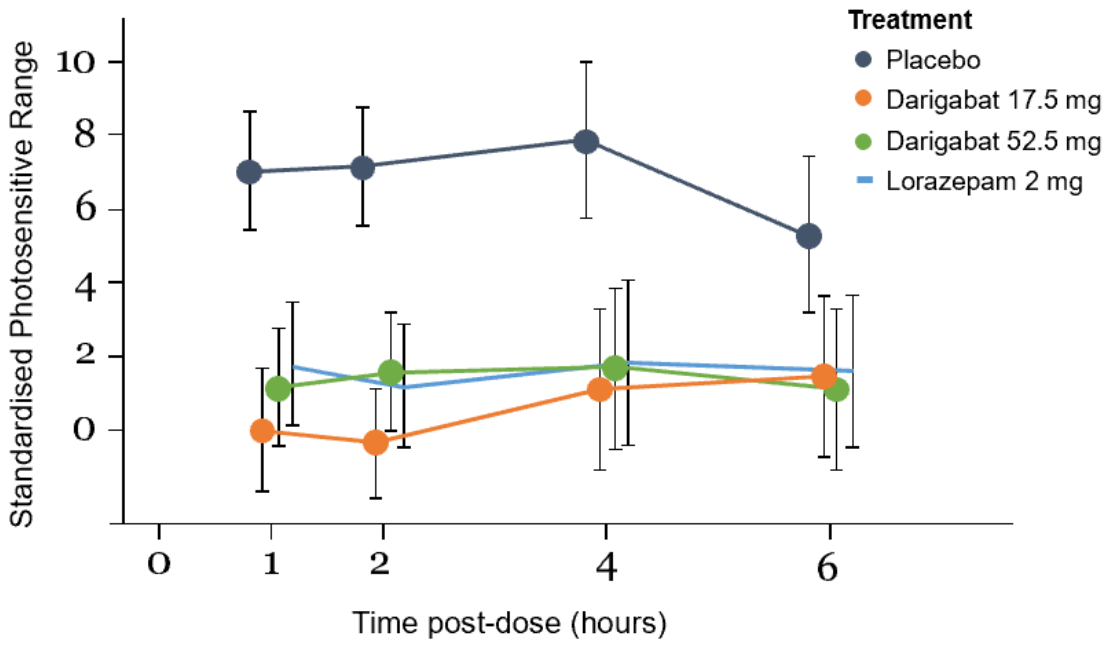
To aid recruitment given that only 3-5% of people with epilepsy are photosensitive, and most are adolescents, only expert clinical sites who had previously participated in photoepilepsy studies were selected for the trial. The trial began with screening to identify whether their patient with documented photoepilepsy met the minimal requirement for photosensitivity as outlined in the eligibility criteria of the trial protocol. All intermittent photic stimulation (IPS) assessments were performed using the systematic protocol previously described by Kasteleijn-Nolst Trenite et al., 1996 and in Gurrell et al., 2019. A standardised EEG photic stimulation procedure was utilised with intermittent white flashes produced by a photic stimulator with at least a 5 second pause between the various flash frequencies (2, 5, 8, 10, 13, 15, 18, 20, 23, 25, 30, 40, 50, and 60 Hz) in 3 eye conditions (eye closure, eyes closed, and then eyes open, and always in this order). To determine the upper and lower IPS thresholds, each frequency was assessed in all eye conditions, starting at 2 Hz. As soon as generalised EEG epileptiform activity appeared, the stimulation for that particular frequency in a particular eye condition was stopped. The frequency at which the first PPR occurred was considered the lower bound of the frequency range for that eye condition. Similar assessments were performed starting at 60 Hz and descending through the standard frequencies. As before, the stimulation was terminated if a generalised response was observed, and the sequence was not continued beyond that frequency in that particular eye condition. This was considered the upper bound of the range at which the subject was photosensitive. The patient is assumed to be sensitive to all the frequencies between the upper and lower bound, however are not tested at these frequencies so as not to precipitate the clinical manifestation of a seizure. The standardised photosensitivity range (SPR) is defined as the number of frequency steps between and including the lower and upper bound at which generalised EEG epileptiform activity has occurred, with a minimum of SPR being 0 and a maximum being 14. Two independent reviewers analysed the data and determined the sensitivity ranges in a blinded fashion.

The primary endpoint was the average least squares mean change in the SPR (as measured by the independent review) in the participant's most sensitive eye condition, across all time points. A reduction in SPR indicated a reduced response to IPS and

indicative of anticonvulsant activity. Secondary endpoints included an analysis of the number of patients who had complete suppression of SPR (defined as a treatment period having SPR=0 in all 3 eye conditions at the same time point), partial suppression (reduction of SPR of at least 3 units from baseline for at least 3 time points), and no time points with at least 3 units of increase, in the most sensitive eye condition, without meeting the complete suppression definition. No response was defined as not meeting the definitions for complete suppression or partial response.

Single oral doses of darigabat 17.5 mg and 52.5 mg had statistically significant greater effect on the reduction of SPR versus placebo, this was also the case for lorazepam (**Figure 3-5**). The overall least square mean of darigabat 17.5 mg response relative to placebo in SPR was -6.2 (90% confidence interval [CI] l -8.6 to -3.9), for 52.5 mg it was -5.4 (90% CI -7.8 to -3.1) and for lorazepam was -5.2 (90% CI -7.6 to -2.8). The statistical probability of having a larger reduction of SPR than placebo was 100% for both doses of darigabat (decision criterion 1). The statistical probability of the reduction of SPR being larger than the target value of 3.2 (half of the average baseline across all participants and treatment periods, criterion 2) was 98% at the 17.5 mg dose, and 94% at the 52.5 mg dose. Regarding the secondary endpoint, the majority of participants (6 out of 7) had complete suppression of the SPR when they received treatment with either a dose of darigabat or lorazepam, in comparison to 5/7 of patients having no response when receiving placebo. There was no apparent difference between the efficacy of low dose of darigabat (which achieves approximately 60% RO), the high dose of darigabat (~80% RO) or lorazepam. This is likely in part due to the floor effect of the model, with all 3 active treatments reaching maximum effect which was sustained throughout the 6-hour time period that was examined. The RO of darigabat is predicted to remain > ~ 45% at the 6-hour timepoint, potentially suggesting that this level of RO is enough to derive some anticonvulsant benefit, unless there is some prolongation of the effect that does not align with the PK properties. However, it would have been interesting to record SPR for a longer period after treatment administration to develop some understanding of the pharmacodynamic/pharmacokinetic (PKPD) profile in this model.

Figure 3-5 Time course of treatment effects for the standardised photosensitivity range (SPR)



Effect of single oral doses of darigabat, positive control lorazepam and darigabat (17.5 mg and 52.5 mg) on the photosensitivity range in patients with documented photosensitive epilepsy. Modelled least square means (LSMean) come from the primary analysis mixed-effect model for repeated measures, analysed used a fixed effect model with statistical significance set at P = 0.05 (one-sided). Data shown in LSMean and 90% confidence interval. Adapted from Gurrell et al., 2019.

Another limitation of this data is that although it has been shown to be predictive of anticonvulsant activity, due to differences in the clinical trial methodologies and the way data has been previously reported from these trials, data from photoepilepsy cannot be used to quantitatively predict the level of reduction of seizures in patients participating in traditional epilepsy clinical trials. This is an important caveat given the large number of AEDs available, and the need to be differentiated from those drugs already approved in the indication.

However, the robustness of this data and the apparent predictivity of the model to anticonvulsant efficacy in wide epilepsy populations was viewed as providing sufficient

confidence in the rationale to embark on further clinical trials in epilepsy patients. This positive data also directly resulted in this program continuing to be part of the neuroscience portfolio at Pfizer despite the neuroscience group being primarily focussed on identifying drugs to treat Parkinson's disease and schizophrenia. When Pfizer announced the exit of early neuroscience research, the photoepilepsy data with darigabat and ability to move into Phase 2 trials with confidence was an enticing value proposition to bring investors, biotechnology, and pharmaceutical companies to the negotiation table. The forward development outlined at Pfizer, to initiate a Phase 2 trial in patients with drug-resistant focal epilepsy has been realised at darigabat's new owner, Cerevel Therapeutics.

3.3.3 Phase 2 trial in patients with generalised anxiety disorder (GAD)

Data from mouse models with mutations which confer specific subunits insensitive to BZDs (Rudolph et al., 1999) have indicated that the α2/3-containing receptors are responsible for the anxiolytic effects of BZDs. As such, a concerted effort has been made to develop the next generation of $GABA_A$ receptor subtype-selective anxiolytic modulators. A compound that moved into clinical development for anxiety was α2/3-selective TPA023, which had been shown to possess a non-sedating anxiolytic-like profile in rodent and non-human primate nonclinical studies (Atack, 2010). Furthermore, a post-hoc analysis of three separate Phase 2 studies in GAD, all of which were prematurely halted due to nonclinical toxicity, demonstrated that TPA023 induced significant decreases in the Hamilton Anxiety (HAM-A) scale compared to placebo (Atack, 2009). The positive data in the combined trial gave proof-of-concept that selectively targeting α2/3-containing $GABA_A$ receptors is anxiolytic. The TPA023 clinical studies in anxiety patients employed flexible dosing schedules of either 1.5-4.5 mg or 3-8 mg total daily doses of an extended release formulation, and predicted occupancies are wide-ranging, potentially achieving 40-70% RO. No further information is publicly available as to whether larger improvements on HAM-A were associated with higher RO.

Based on both in-house positive data in nonclinical models of anxiety with darigabat (Owen et al., 2019), and the clinically precedented positive data with TPA023 in patients with GAD (Atack, 2009), a double-blind, randomised, placebo-controlled Phase 2 trial

designed to evaluate the effect of darigabat on patients with GAD was conducted (Simen et al., 2019; note the author was not involved in the design or execution of the GAD trial). The trial was terminated early by Pfizer for project prioritisation reasons when a total of 90 patients had been randomised into the trial (of the planned 384). However, analysis of the available data showed that neither the 2.5 mg BID nor 7.5 mg BID doses of darigabat differentiated from placebo on week 4 HAM-A scores, although there was evidence of pharmacology in both the Digit Symbol Substitution Test (DSST) and Epworth Sleepiness Scale (ESS) (Simen et al., 2019). Factors contributing to the lack of anxiolytic effect include the limited sample size, and the potentially subtherapeutic doses evaluated, which achieved at maximum, approximately 60% occupancy. In addition, this trial enrolled patients with treatment-resistant anxiety, defined as persistent symptoms of anxiety despite treatment with background standard of care therapy. The selection of this particularly treatment-resistant patient population may have contributed to a negative result. As such, the anxiolytic potential of darigabat is yet to be fully evaluated. Unsurprisingly, there is a lack of desire to embark on a large and expensive trial in GAD at higher doses than previously examined and as such, an experimental medicine approach was identified to evaluate the anxiolytic potential of darigabat at > 50% RO and this is discussed in **Chapter 5.2.3**.

3.4 Discussion

Nonclinical studies demonstrate that darigabat has analgesic, anxiolytic, and anticonvulsant efficacy in rodent models. In three Phase 2 clinical trials in pain, anxiety, and epilepsy, darigabat has demonstrated efficacy only in patients with epilepsy. Despite the obvious contributions that animal models have made to our understanding of pathobiology and the fact that new drugs have been developed based on efficacy in animal models, substantial criticism has been imposed for their lack of clinically predictive value. Whilst it is tempting to blame the animal models of pain and anxiety for a lack of predictive validity to the clinic for darigabat, issues with translation and attrition in clinical development are not solely due to problems arising from the use of nonclinical models. There may, for example, be confounding factors associated with the basic science, the program strategy or decision-making process, in the selection of patient population or in

the clinical trial design, dose selection, and one or multiple elements are likely responsible for the disappointing data with darigabat in pain and anxiety.

Due to a lack of suitable nonclinical models of back pain, a model of neuropathic pain in rats was used as a surrogate and demonstrated analgesic potential of darigabat at ~ 70% RO. Although a lack of back pain model is far from ideal, a comparison of the PKPD relationship for pain drugs in rats and humans concluded that overall, the rat predicts efficacious drug exposure for approved analgesics of differing mechanisms across models of acute, inflammatory, and neuropathic pain (Whiteside et al., 2008). Whilst the authors considered a 10-fold difference between species (rat to human) indicative of predictive utility, there was considerable variation between individual compounds, with some compounds showing a close correlation but for others it was not (with 50-fold being the worst observed translational ratio). Clearly, a shift of magnitude within a species would be considered problematic and where darigabat lies on this spectrum remains unascertained.

Evidence of analgesic potential of single doses of darigabat was observed in the PainCart trial in which PKPD were examined using a multi modal battery of pain challenges in healthy participants (van Amerongen et al., 2019). Analgesia was observed in a limited number of pain challenges and the effect size was pregabalin > darigabat 65 mg > darigabat 15 mg, suggesting that, particularly at the lower explored dose of 15 mg which results in approximately equivalent concentrations to the 7.5 mg BID dose selected for the CLBP trial, analgesic potential was modest. Should the data from the PainCart trial have been available prior to initiating the CLBP trial it is tempting to speculate that the latter may have been stopped or paused, at least until the self-imposed dose cap which limited multiple dose studies to ≤ 7.5 mg BID had been lifted. However, the clinical trial in CLBP with darigabat was successful in yielding clear results and supports the notion that significant antihyperalgesia may only be obtained in humans at very high RO with this MOA. The highest dose examined in this trial (7.5 mg BID) achieved a maximum of approximately 60% RO (**Table 2-1**). Although a single dose of 15 mg darigabat demonstrated a robust effect on SPV (-65 to -73 degrees/sec) which exceeded that of a single dose of lorazepam 2 mg (-39 degrees/sec), it is roughly half the maximal change

in SPV observed with darigabat at singles doses of 65 and 100 mg (~ -130 degrees/sec) which achieve > 80% RO (Nickolls et al., 2018). As such, the effect of darigabat in pain remains unknown at doses that achieve maximal PD impact and RO as these dose ranges have yet to be explored in this indication.

Both clinical and nonclinical data have indicated the potential for α2/3-selective modulators, such as darigabat, to be anxiolytic. However, studies to date have generated conflicting findings (e.g., ocinaplon, AZD7325, and darigabat), whereas other compounds (e.g., NS1139 and TPA023) have yielded data consistent with this hypothesis (Atack et al., 2009). There were several significant limitations of the GAD trial with darigabat. The trial was underpowered as a consequence of the early termination of the trial, meaning that only 90 of the planned 384 patients were randomised. Nevertheless, the fact that patients receiving darigabat had numerically worse HAM-A scores compared to patients receiving placebo would suggest that darigabat is unlikely to be effective in the selected patient population at the doses explored. This trial enrolled only patients with treatment-resistant anxiety, defined as persistent symptoms of anxiety despite treatment with adjunctive standard of care therapy, such as with selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors. Since 1990 no drugs anticipated to treat mood and anxiety have been approved for use in these patients in the US and treatment-resistant populations are historically very difficult populations in which to demonstrate efficacy which could reduce the likelihood of seeing treatment effect in the setting of a clinical trial. In common with the CLBP trial, the highest dose examined in this trial (7.5 mg BID) was unlikely to test the mechanistic hypothesis and given that this is submaximal with respect to SPV and RO, the effect of darigabat and the ultimate role of the α2-, α3-, and α5-containing $GABA_A$ receptors in anxiety disorders remains unknown.

Although darigabat failed to meet the primary endpoint in both the CLBP and GAD trials, there was evidence of pharmacology in both studies suggesting that although 60% RO is not analgesic or anxiolytic in the patient populations selected, it does induce pharmacological activity through the duration of the 4-week administration period. In CLBP there was evidence of improvement in physical function as measured by the RMDQ and some impairment in cognitive performance in the HVLT-R in those randomised to

receive darigabat. In GAD, those administered 7.5 mg BID darigabat showed some evidence of worse performance on the DSST and ESS. These observations are important as the lack of analgesia or anxiolysis may be interpreted as a result of tolerance, but the secondary endpoints examining the pharmacology of darigabat would suggest otherwise. However, it cannot be ruled out that the different α subunit subtypes of $GABA_A$ receptors have differing sensitivities to tolerance, as exemplified by administration of BZDs results in tolerance in epilepsy > anxiety (Gravielle, 2016). The current POC and 57-week open label extension trials with darigabat in patients with focal epilepsy will provide more definitive data in support of the concept that a subtype-selective PAM with lower functional efficacy than a BZD has a lower propensity for tolerance (see **Chapter 5**).

In contrast to both the CLBP and GAD trials with darigabat, the Phase 2 photoepilepsy trial not only demonstrated robust efficacy, but also indicated that only partial (~ 60% RO) occupancy is required for activity (Gurrell et al., 2019). In the photoepileptiform trial both single doses of 17.5 mg and 52.5 mg darigabat resulted in full-abolition of the IPS-evoked PPR in 6 out of 7 patients. There was no dose-response relationship observed in this trial with both doses showing similar levels of maximal efficacy, which may have been a result of the floor effect in the model. The data reported are consistent with a wide acknowledgement that $GABA_A$ receptors play a role in convulsant pathways, although some inconsistencies regarding relative contributions of the $GABA_A$ receptor α subunits to the anticonvulsant activity of BZDs have been reported. For example, the anticonvulsant efficacy of diazepam has been ascribed primarily to the α1 subunit in molecular studies in mice with α subunits rendered insensitive to BZDs (H101R mutants; Rudolph et al., 1999). However, comprehensive work using both subtype-selective $GABA_A$ receptor PAMs together with transgenic mice with point mutations altering the BZD-binding site at other $GABA_A$ receptor subtypes (α2-H101R, α5-H105R) demonstrated that no single receptor subtype is solely responsible for the anticonvulsant effects of GABA (Fradley et al., 2007). Based on that work it was reported that the α2 subunit played a greater role than the α1-containing receptors, concluding that efficacy at more than a single $GABA_A$ receptor subtype can be achieved and that the α1- and α2-containing receptors act synergistically, at least in animal models. As demonstrated by

both the nonclinical and clinical data with darigabat in epilepsy, it is reasonable to conclude that the α2 subunit is important in conferring the anticonvulsant activity of BZD-sensitive $GABA_A$ receptors. However, given the high binding affinity of darigabat at α1-containing receptors and low (~20%) but not absent functional efficacy via this subunit (**Table 1-4**), a role of the α1 subunit in anticonvulsant efficacy cannot be ruled out.

It is not clear why only partial RO may be required to achieve efficacy in this epilepsy measures as compared to higher RO levels in pain or anxiety disorder in humans. It could be related to the different combinations of subunits contributing to the efficacy in the different indications, with potentially a more prominent role of the α1 subunit in the anticonvulsant activity of this mechanism compared to that in anxiety or pain. Alternatively, it could be due to key differences in the trial designs between the different indications. For example, the photoepilepsy trial utilised a single dose and a non-registrational endpoint in contrast to trials in pain and anxiety where sustained benefit is being measured. Whether higher RO is required in multiple dose future clinical trials in epilepsy to incur clinically meaningful benefit is yet to be determined.

The data from Phase 2 clinical trials with darigabat to date have been both disappointing and informative. Each trial has significantly contributed to our understanding of selectively targeting subtype-selective $GABA_A$ receptor PAMs to treat a range of indications and has shaped the current drug development strategy for the darigabat program.

3.5 References

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4 SAFETY

4.1 Introduction

Benzodiazepines (BZDs) are highly efficacious in epilepsy and anxiety disorders but have significant side effects that limit their clinical utility in these clinical indications. For example, BZD use is often associated with dose-limiting sedation, somnolence, and cognitive impairment, along with the risk of development of physical and psychological dependence. Many of the negative attributes of BZDs have been ascribed to the α1-containing $GABA_A$ receptors and it has therefore been a clear objective of the darigabat program to demonstrate that targeted pharmacology which minimises activity at α1-containing receptors is associated with a differentiated nonclinical and clinical safety profile compared to non-selective BZDs.

An understanding of the safety profile of darigabat has been obtained from regulatory toxicity studies in nonclinical species, adverse event reporting in clinical trials, plus the use of patient-reported scales to assess withdrawal symptoms associated with discontinued administration of darigabat. The contribution of the author to these works is outlined in **Table 1-1** in **Chapter 1**.

4.2 Nonclinical safety studies

4.2.1 Nonclinical toxicology studies

Darigabat has been evaluated in several safety pharmacology studies, genotoxicity studies, embryofetal developmental toxicity studies in rats and rabbits, fertility studies in male and female rats and an *in vivo* phototoxicity study (data unpublished). Six- and nine-month multiple-dose toxicology studies have been conducted in rats and dogs, respectively. The nonclinical data packages support long term clinical studies at a dose of 25 mg BID, including the 57-week open label extension study in patients with focal epilepsy that is currently ongoing. In reproductive toxicology studies, effects on rats and rabbits included malformations that are consistent with a requirement for contraceptive practice to be in place in patients treated with darigabat, which is consistent with many other approved antiepileptic drug (AED) label warnings.

Beyond the regulatory requirements to understand the safety and exposure profile nonclinically and aid clinical dose selection, observations in these studies can help

understand the on- and off-target pharmacology of the compound of interest. For darigabat, two observations that were suggestive of a differentiated profile of darigabat versus BZDs were noted in the chronic toxicology studies. First, behavioural observations related to GABAergic modulation in dogs at supratherapeutic doses are maintained for the 9-months of administration (data unpublished). Whilst this provides some supportive data to indicate that darigabat is devoid of the tolerance effects that are problematic with BZDs, it does not however provide evidence directly related to lack of efficacy tolerance in epilepsy, for example.

Second, abrupt discontinuation of darigabat administration after 6- or 9-month dosing in rats and dogs respectively did not result in severe withdrawal symptoms such as seizures. However, relatively short-term (2-week) administration of the BZDs diazepam and lorazepam in dogs followed by abrupt discontinuation has been reported to result in severe abstinence syndrome indicating that there was physical dependence (McNicholas et al., 1983). Whilst these observations are encouraging, specific nonclinical studies to examine abuse potential and withdrawal of darigabat are yet to be conducted. The United States Food and Drug Administration (FDA) recommends conducting these typically after the end of Phase 2 clinical trials, when the final therapeutic doses are more fully predicted as the doses selected for testing in animal abuse-related studies should be based on achieving equivalent unbound plasma levels to those observed in humans at the highest proposed therapeutic dose.

In early nonclinical genotoxicity studies, darigabat was identified as a potential aneugen (Owen et al., 2019), a substance that causes a daughter cell to have an abnormal number of chromosomes, and results in observations of micronuclei (MN), small nuclei that form whenever a chromosome or fragment of a chromosome is not incorporated appropriately into a daughter nucleus during cell division. It is essential to elucidate if the MN induction is via a clastogenic or aneugenic mechanism because clastogens that directly induce DNA damage may not have a threshold effect (so it is rarely possible to set safety margins for genotoxicity). Aneugens, however, do not target DNA directly but hamper the functions of nuclear mitotic apparatus, such as spindles and tubulin filaments. Microtubule impairment affects the rate of division of the fast-diving cells such as those present in the

gut or white blood cells for example, resulting in the clinical manifestation of diarrhoea or neutropoenia, respectively. As such, the resultant effects are clinically monitorable and typically reversible on cessation of drug administration. The results of the tubulin polymerisation assay (data unpublished) demonstrated that darigabat may destabilise the formation of microtubules at supra-therapeutic exposures.

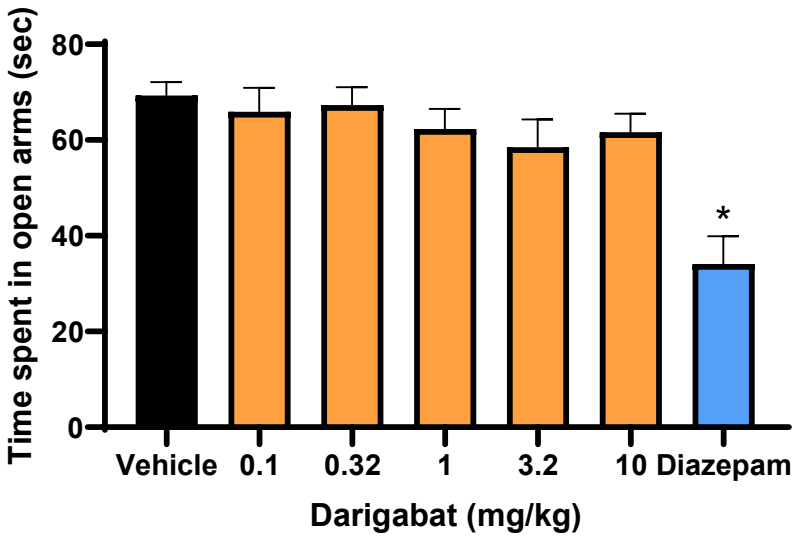
The current International Conference Harmonisation (ICH) Guidance on Genotoxicity Testing states that compounds that induce aneuploidy do so only within a narrow range of doses that are approaching toxic doses and associated endpoints can be measured in *in vivo* MN assays in bone marrow or blood assays (ICH Guidelines Genotoxicity Testing 2012). As such, aneugenicity is not a barrier to drug development as the effect is well understood with a clear exposure threshold, whilst duration of exposure has no impact on aneugenicity or clinical safety when staying below the exposures threshold (Muller and Kasper, 2000; Thybaud et al., 2007). However, prior to initiating multiple dose trials with darigabat, an arbitrary dosing cap was imposed of 1/10th of the exposure at which MN were observed *in vivo*, resulting in a maximum dose of 7.5 mg BID (approximately 60% receptor occupancy (RO)) being permitted in repeated dose clinical trials. Subsequent discussions with FDA resulted in the arbitrary dose cap being removed, and this is discussed in **Chapter 4.3.2**.

4.2.2 Nonclinical rotarod study

BZDs are known to impair motor coordination and clinical use is associated with an increase in patients falls, particularly in the elderly (Diaz-Gutierrez et al., 2017). In nonclinical research, the accelerating rotarod is used to identify negative effects on motor function. In this nonclinical evaluation a rodent is placed on a rotating cylinder above a cage floor and the rodents naturally attempt to stay on the rotating cylinder and avoid falling. The length of time the animal stays on (capped at 2 minutes) can be used as an assessment of their motor coordination. The effect of oral darigabat (0.1-10 mg/kg), diazepam (10 mg/kg), and vehicle were evaluated in the mouse accelerating rotarod approximately one hour after dosing in rodents acclimatised to the procedure. Time to fall was significantly decreased in mice treated with diazepam, but not for mice treated with

darigabat compared to vehicle treatment (Bialer et al., 2020), indicating a less impairing effect of darigabat, even at maximal RO (**Figure 4-1**).

Figure 4-1 The effect of darigabat on fall latency on the accelerating rotarod in mice



Time to fall from an accelerating rotarod was assessed at 1-hour post oral administration. 10 mg/kg diazepam was used as the positive control. Data are means ± standard error of the mean, n=12 per group. * = P<0.05, one-way ANOVA comparing all doses to vehicle followed by unpaired t-test comparing vehicle to diazepam treated group. (Bialer et al., 2020).

4.3 Clinical studies

Darigabat has been administered to 289 participants in 9 completed clinical trials (Bialer et al., 2020) to date and an additional number of participants have or are receiving darigabat in the 3 ongoing clinical trials (see **Chapter 5**). Darigabat has been administered up to 100 mg as a single dose (Nickolls et al., 2018) and up to 42.5 mg in multiple doses (Gurrell et al., 2021). To date, darigabat has been generally safe and well tolerated with the majority of adverse events (AEs) being mild-moderate in severity. Across trials there have been no clinically significant observations related to tubulin-binding, such as diarrhoea or neutropenia.

Across 6 completed Phase 1 trials, a total of 81 healthy participants received single doses and 55 healthy participants received multiple doses of darigabat for up to 21 days. Darigabat was assessed to be generally safe and well tolerated with no clinically significant safety observations in this population. The most common AEs following darigabat were dizziness, somnolence, fatigue, and bradyphrenia. All AEs were mild or moderate in severity. There were no drug-related trial withdrawal events and no treatment-related serious AEs reported across the Phase 1 trials.

In 2 completed Phase 2 trials, a total of 146 participants, 74 with chronic low back pain (CLBP) and 72 with generalised anxiety disorder (GAD), received multiple doses of up to 7.5 mg BID of darigabat for up to 4 weeks (Gurrell et al., 2018; Simen et al, 2019). Additionally, 7 patients with photosensitive epilepsy were enrolled in the single dose Phase 2 trial (Gurrell et al., 2019). No clinically significant safety findings emerged across the Phase 2 trials. The most frequently reported AEs following darigabat treatment were dizziness and somnolence and most were mild or moderate. Use of a titration in the multiple dose studies appeared to decrease the incidence of CNS AEs. Four participants, all in the darigabat groups, discontinued treatment due to AEs, three of which were assessed not to be treatment-related. One participant experienced a serious AE (transient ischaemic attack) that was determined by the investigator to be related to darigabat; this participant had a history of multiple cardiovascular risk factors and was subsequently diagnosed with Type 2 diabetes mellitus (Gurrell et al., 2018).

There have been no self-reported AEs related to withdrawal symptoms in any trial, even following administration of multiple dosing up to 42.5 mg BID, or as measured by the Physician's Withdrawal Check list in pain (Gurrell et al., 2018; Gurrell et al., 2021) and anxiety patient trials (Simen et al., 2019), notwithstanding the exposures in those trials may be subtherapeutic.

The current clinical safety data with darigabat has shown that it is generally safe and well tolerated. Key differentiators from α-subunit non-selective BZDs are the lack of sedation, even following single doses of 100 mg which achieve saturating levels of RO.

4.3.1 First-in-human trial

The first-in-human (FIH) clinical trial is a pivotal moment in any research and development program. Whilst the proportion of drugs failing due to poor pharmacokinetic (PK) or bioavailability properties fell to 10% following the introduction and widespread implementation of the “Lipinski Rule of Five” (Lipinski et al., 1997), there continues to be a significant proportion (~30%) of drugs that fail due to safety (Kaitin and DiMasi, 2011). Subtype selective $GABA_A$ receptor positive allosteric modulators (PAMs) have not been immune to this failure, with the translation of minimal α1-potentiation *in vitro* to a lack of dose-limiting sedation or somnolence in the clinic not always proving to be the case (see **Chapter 2.2.3**). Given that darigabat exhibited no known differentiable nonclinical attributes from, for example, TPA023, it was unknown whether the clinical experience of darigabat would be divergent from the dose-limiting drowsiness observed with TPA023 (Atack, 2010), highlighting the importance of the FIH trial.

Accordingly, the single ascending dose FIH trial was designed to examine PK, safety, tolerability, and pharmacodynamic (PD) effects of darigabat using NeuroCart in healthy participants and is described in detail in Nickolls et al., 2018. The doses explored in the trial ranged from 0.04 to 100 mg in four cohorts, with the fourth cohort including lorazepam 2 mg to enable comparisons of PD effects with a non-selective BZD (data currently unpublished). The PD effects as measured by NeuroCart are described in **Chapter 3.2.3**.

In summary, single oral doses of darigabat escalating from 0.04 to 100 mg were generally safe and well tolerated. The most frequently reported treatment-emergent adverse events (TEAEs) were dizziness (up to 75%), somnolence (up to 63%), bradyphrenia (up to 50%), elevated mood (up to 38%), fatigue (up to 25%), headache (up to 25%), and orthostatic hypotension (up to 38%), (see Supplementary Table 2 in Nickolls et al., 2018 for more details). Notably there were no reports of sedation at any dose. Overall, a greater number of TEAEs were experienced by participants in the higher dose groups compared to the lower dose groups. However, all TEAEs were mild in severity and the severity of AEs did not increase with increasing doses beyond 6 mg. It is possible that the AEs could be in part associated with potentiation of α1-containing receptors by darigabat. Although darigabat has minimal functional efficacy at α1, it has a K_i of 0.18 nM at α1-containing

receptors, 16-fold lower than at α2 (Nickolls et al., 2018). Thus, the α1-containing receptors will be preferentially bound at lower doses, with significant α2 binding and efficacy occurring at relatively higher doses. This is supported by data from the PD biomarker measured by NeuroCart body sway in which small, but significant increases were observed at sub-milligram doses which then plateaued at approximately 10 mg without further increase up to 100 mg despite marked increases in RO across this dose range.

There were no deaths, serious, or severe AEs, discontinuations, or temporary discontinuations due to AEs. There were no clinically significant observations on laboratory assessments, vital signs, or ECGs and a maximum tolerated dose was not achieved with 100 mg. Escalation beyond 100 mg was not possible due to safety margins based on nonclinical regulatory toxicological studies.

Whilst the majority of data from the fourth cohort are not yet published, Figure 7 of Nickolls et al., 2018 plots the TEAEs of dizziness and somnolence for the single oral doses of darigabat in cohorts 1-3 and of lorazepam 2 mg in cohort 4. Comparatively, lorazepam administration results in lower rates of dizziness (20%) versus doses of darigabat > 6 mg (rates of up to 75%), and similar rates of somnolence to doses 6 – 100 mg of darigabat (approximately 53% with lorazepam and up to 63 % with darigabat). Whilst this data does not appear to show any differentiation between darigabat and lorazepam in this FIH there are some key observations which are suggestive of a distinct profile of darigabat. For example, not only were no dose-limiting AEs observed with darigabat, but the severity of CNS AEs did not increase with increasing doses. In contrast, single high doses of BZDs can result in profound CNS depression ranging from mild drowsiness to a coma-like, stuporous state (Kang et al., 2020), even at ~ 20% RO (Atack, 2010). The subsequent positron emission tomography (PET) trial later confirmed that the RO at the highest doses exceeded 80% (Nickolls et al., 2018). Furthermore, even some α1-sparing $GABA_A$ receptor PAMs have failed in FIH due to sedation or dose-limiting drowsiness (Atack et al., 2011). Overall, the data from this trial demonstrated that further clinical investigation of darigabat was warranted.

4.3.2 Multiple ascending dose trial

Two multiple ascending dose (MAD) studies have been performed with darigabat. The first was performed when the self-imposed dosing cap was in place and examined the safety, tolerability, and PK of ascending repeated doses over a 2-week period (Clinical Trials ID NCT02070289, data unpublished). Doses examined ranged from 2.5 mg BID to 12.5 mg BID, however the dose of 7.5 mg BID was selected for subsequent multiple dose trials in pain and anxiety to maintain the concentrations at 1/10th of the exposure at which MN were originally observed *in vivo* (see discussion above in **Chapter 4.2.1**).

The ability to fully characterise the potential of darigabat in the clinic had been potentially hampered by the multiple dose cap which limited plasma exposure to concentrations which achieve approximately 60% RO. The therapeutic potential of this mechanism of action in both pain and anxiety disorder remains unknown given the potentially subtherapeutic doses used in the CLBP (Gurrell et al., 2018) and GAD (Simen et al., 2019) trials. Whilst the single dose photoepilepsy trial had been positive at the lower 60% RO (Gurrell et al., 2019), epilepsy is a highly competitive area in which a drug needs to show sufficient efficacy to be both approved by the regulatory agencies, be accessible to patients via medical insurance companies in the US, or to be considered early in the treatment paradigm in UK/Europe. Given that > 60% RO of darigabat is associated with an increased PD effect on saccadic peak velocity (SPV) via α2/3 subunit-containing $GABA_A$ receptors (Nickolls et al., 2018), it is reasonable to presume that increased RO in patients with epilepsy may result in increased efficacy.

Therefore, advice was sought from the FDA via a Type C meeting request regarding increasing multiple doses beyond the arbitrary self-imposed dose cap and if they had any objections to the proposed clinical doses and endpoints. A draft clinical protocol was provided to FDA describing an additional MAD trial in which doses of up to 50 mg BID were to be administered to healthy participants and PK, safety, and tolerability were to be examined. In addition, an exploratory endpoint to assess changes in MN frequency compared to pre-dose was proposed. MN expression in peripheral blood lymphocytes is well established as a standard method for monitoring chromosome damage in humans, and specifically, human blood micronucleated reticulocyte (MN^{CD71+}) frequency

measurement may be used a marker of cytogenetic damage (Dertinger et al., 2007). As such, MN^{CD71+} frequency was selected as an exploratory clinical biomarker as whilst not used routinely in clinical trials, it is used routinely in nonclinical research and represents the best available quantitative marker of aneugenicity. Feedback was received from FDA in 2017 that darigabat may administered up to 50 mg in multiple dose trials and that the previous arbitrary dosing cap was not necessary. Therefore, the healthy participant protocol to examine higher than previously examined multiple doses of darigabat was finalised and initiated.

The design and results of this trial are described in detail in Gurrell et al., 2021, with the exception of the exploratory MN data which are summarised here. Briefly, a total of 19 healthy adult participants were enrolled into 2 successive cohorts. Within each cohort participants were randomised to receive blinded darigabat or placebo. The 2 doses selected to investigate in the trial were chosen to limit exposure to below the threshold for aneugenicity in rats at which MN were observed (unbound maximum plasma concentration [C_{max}] of 150 ng/mL). The 25 mg BID and 42.5 mg BID doses were projected to result in unbound C_{max} values of 28 ng/mL and < 57 ng/mL respectively, which equated to approximately one-fifth and one-third of the no observed effect level (NOEL) for MN formation in the rat.

The timing of the potential maximal effect of darigabat on MN^{CD71+} frequency was unknown; therefore, measurements were obtained on multiple days (Days 4, 8, 11, 14, and 21) during darigabat administration. A follow-up measurement was also obtained 7 to 10 days after the last dose to confirm reversal of any potential increases in MN^{CD71+}. Samples were analysed by Litron Laboratory using flow cytometry in conjunction with a 3-colour fluorescence labelling technique that included anti-CD71 to focus on analysis of MN^{CD71+} (Dertinger et al., 2004; Dertinger et al., 2007).

Formal dose-escalation stopping rules were defined in the protocol based on safety and tolerability parameters and exposure criteria designed not to exceed the human dose exposure limits. Although no formal stopping rules were stipulated related to MN^{CD71+} due to the exploratory nature of the endpoint, they were to be used to further understand benefit:risk of potential doses in future clinical trials, with an expectation that MN^{CD71+}

values from 50 healthy participants ranged from 0.04% to 0.28% (mean, 0.12%) (Dertinger et al., 2007).

In 2 cohorts (7-8 administered darigabat and 2 administered placebo in each cohort), healthy adult participants received twice-daily oral doses of darigabat for 21 days. Darigabat was titrated to a maximum dose of 25 mg BID in Cohort 1 (5 mg BID for 3 days, then 12.5 mg BID for 4 days, then 25 mg BID for 14 days) and to a maximum dose of 42.5 mg BID in Cohort 2 (5 mg BID for 2 days, then 12.5 mg BID for 2 days, then 25 mg BID for 3 days, then 42.5 mg BID for 14 days), with the total duration of treatment being 21 days. Serial PK samples were collected on Days 1 and 21.

An overview of the PK and safety results are detailed in Gurrell et al., 2021. Briefly, approximate dose-proportional increases in C_{max} and area under the curve (AUC) over the time period tested were observed and darigabat was rapidly absorbed with a median T_{max} of 1 to 2 hours following both single-and multiple-dose oral administration (Figure 1 and Table 1 in Gurrell et al., 2021). Mean t½ on Day 21 was approximately 11 hours in both cohorts. All AEs were mild; the most frequently reported was dizziness (reported by 25% of participants administered placebo, 25% of participants administered 25 mg darigabat, and 42% of participants administered 42.5 mg darigabat) (Figure 4 and 5 in Gurrell et al., 2021). Post-titration, there were no reports of somnolence and importantly, there were no reports of sedation by any participant. There were no clinically significant safety findings, including no changes in neutrophils and a lack of withdrawal symptoms on discontinuation of treatment.

Of the 19 participants who were enrolled in the trial and randomised, 18 participants had a least 1 value for MN^{CD71+}. No MN^{CD71+} data were reported for 1 of the 4 placebo-treated participants. The results of the analysis of the exploratory clinical biomarker demonstrate that twice-daily administration of 25 mg or 42.5 mg of darigabat for 21 days (7 days of titration, followed by 14 days at the target dose) did not result in an increase in MN^{CD71+} frequency relative to baseline at any timepoint tested (data unpublished). MN^{CD71+} frequency varied little from baseline in either the darigabat treatment groups over the trial and the pattern and magnitude of change in MN^{CD71+} frequency in the darigabat treatment groups were similar to those in the placebo group throughout the trial. The results of this

exploratory clinical biomarker indicate that darigabat did not increase MN^{CD71+} frequency. This lack of response suggests that darigabat was not aneugenic at doses of 25 mg BID and 42.5 mg BID, as predicted by the nonclinical data which suggests that these clinical exposure levels are below the threshold to potentially induce aneugenicity.

This trial generated key data to support continued clinical development of darigabat at higher doses than previously examined in other multiple dose trials. The exposures achieved in this trial resulted in estimated RO at $GABA_A$ receptors α2 subunits of >80%, which contrasts with RO achieved with BZDs which are limited to <20% to avoid AEs associated with sedation and somnolence in some patient populations (Atack et al., 2011). Indeed, no somnolence was observed in the cohort administered 42.5 mg BID, and only transient, mild somnolence was reported in the 25 mg BID cohort during the titration, providing further evidence of differentiation of darigabat from BZDs. For example, the United States Package Insert for Ativan® (lorazepam), one of the most commonly prescribed BZDs, states that in a sample of approximately 3500 patients treated for anxiety, the most frequent adverse reaction was sedation (15.9%), which will likely equate to doses that achieve receptor occupancy <20%.

An attractive attribute associated with subtype selective PAMs with relatively low intrinsic efficacy versus BZDs, is that there is no convincing evidence that chronic administration leads to symptoms of withdrawal following abrupt discontinuation or the development of tolerance of anticonvulsant efficacy nonclinically, and thus are potentially unlikely to lead to withdrawal or tolerance clinically (Vinkers and Olivier, 2012). This trial adds weight to that evidence as no participant reported any AEs associated with the symptoms of withdrawal following abrupt discontinuation of darigabat. This is in contrast with clobazam, for example, where withdrawal-related AEs were observed in Phase 1 studies following abrupt discontinuation at therapeutic and subtherapeutic dosages (Tolbert et al., 2014).

4.4 Discussion

The current nonclinical and clinical safety data is supportive of the α1-sparing profile of darigabat and the ongoing Phase 1 and Phase 2 clinical trials at multiple doses of up to 25 mg BID. The nonclinical toxicology package highlighted potential *in vivo* aneugenicity

(Owen et al., 2019), as a risk which warrants further monitoring, although the data from the MAD trial at doses up to 42.5 mg BID (for 2 weeks following a 1-week titration) which demonstrated no change in MN^{CD71+} frequency does provide evidence that the risk is mitigated by the doses selected for the ongoing clinical trials. The inclusion of MN^{CD71+}-related endpoints in clinical trials for compounds with aneugenic potential also warrants further consideration. However, based on potential for aneugenicity induced by darigabat *in vivo*, participants with haematologic abnormalities are not currently eligible for the ongoing clinical trials and any clinically significant changes in haematologic parameters during trial participation is designated an adverse event of special interest (AESI) which must be flagged with the medical team for immediate review as a precautionary measure at this stage of clinical development.

Mechanistically related to the potential aneugenic risk of darigabat *in vivo*, is a potential risk to the unborn foetus of clinical trial participants. As such, there are contraceptive requirements in place in the clinical trials with darigabat for men and women of child-bearing potential who are sexually active with someone of the opposite sex. Risks associated with use of AEDs during pregnancy are a major concern for all women with epilepsy who are of childbearing potential (Tomson et al., 2019) and there is not yet clinical data related to exposure of darigabat in utero.

To date, there have been no reports of sedation related to administration of darigabat at any dose in any clinical trial, and the reports of somnolence and dizziness have been mostly mild. The lack of sedation, even at doses achieving > 80% RO is a key distinguishing feature of darigabat versus BZDs, in which significant sedation is observed at > 20% RO. There have also been no self-reported AEs related to withdrawal symptoms in any trial with darigabat, or as measured by the Physician's Withdrawal Checklist (Gurrell et al., 2019; Simen et al., 2019; Gurrell et al., 2021), although the doses in the patient trials were potentially subtherapeutic. This "clean profile" with respect to withdrawal could be interpreted as a lack of pharmacology following multiple doses, or a development of tolerance. With respect to the former, there was evidence in both the 4-week pain and anxiety disorder trials of some pharmacology related to mildly impaired cognition at 2- and 4-weeks of dosing of darigabat (Gurrell et al., 2018; Simen et al.,

2019), although data from the Phase 2 focal epilepsy trial in which darigabat is administered for a total of 13-weeks will provide more definitive data.

Self-administration studies in nonclinical species have demonstrated that α1-containing receptors significantly contribute to the reinforcing effects and withdrawal symptoms of BZDs (Griffiths et al., 1992; Ator, 2005 and 2010). For example, TPA123 possesses 23% functional potentiation at α1 subunits and was associated with BZD-like drug reinforcement and withdrawal symptoms, whereas TPA023 which possesses comparatively less α1 activity did not (Ator et al., 2010). However, it is still possible that α2 and α3 contribute to self-administration given that L-838417 which has neglible α1 activity contiued to self-administer the compound (Rowlett et al., 2005). Although, a drug-discrimination study has been completed with darigabat using zolpidem as a interoceptive cue (Nickolls et al., 2018), primarily to investigate the pharmacology of darigabat, self-administration studies are yet to be completed in nonclinical species.

The currently ongoing trials have been designed not only to understand the therapeutic potential of darigabat in epilepsy and anxiety disorder, but also to further characterise the safety profile. For example, in the ongoing trials in focal epilepsy, a scale to is being used to quantitatively assess withdrawal symptoms during the taper phase of the trial using a modified version of the Clinical Institute Withdrawal Assessment Scale – Benzodiazepines (mCIWA-B). The mCIWA-B is a sensitive instrument to measure withdrawal under conditions where there is a taper of medication (rather than abrupt discontinuation) (Busto et al., 1989) and data will be used to indicate whether this drug has a lower propensity to induce withdrawal symptoms compared to data reported with BZDs. The epilepsy trials also include an Abuse Potential Monitoring Plan with the objective to monitor for instances of abuse or diversion of the trial medication. This is accomplished by monitoring for irregularities in medication handling and training the participating research sites in identifying potentially abuse-related AEs. Examples of potentially abuse-related AE terms for the darigabat mechanism of action include abnormal behaviour, euphoric mood, feeling drunk, and sedation and are aligned with the FDA guidance of terms suggestive of abuse potential (Assessment of Abuse Potential of Drug, FDA Guidance for Industry, 2017). Whilst gathering this data does not replace the

need for the regulatory required abuse potential studies which will likely be conducted in parallel with Phase 3 clinical trials, they do provide some information to aid the design of those trials.

Overall, the safety data supports ongoing clinical evaluation of darigabat at multiple doses that achieve RO ≥50%, enabling the possibility to fully test the mechanism and develop a fuller understanding of the therapeutic potential of this subtype selective $GABA_A$ receptor modulator.

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5 CURRENT CLINICAL TRIALS AND FUTURE DIRECTION

5.1 Introduction

Darigabat is currently under investigation in clinical trials for both epilepsy and panic, with two ongoing trials in patients with focal epilepsy and one trial ongoing in an experimental model of panic (**Figure 5-1**). Whilst there is a body of evidence that subtype-selective $GABA_A$ receptor positive allosteric modulators (PAMs) have a potential utility in a range of clinical indications, epilepsy and anxiety disorder were selected as the lead and second indications due to the perceived higher confidence in rationale for these versus other indications like, for example, pain. Positive data with darigabat in the photosensitive epilepsy model was pivotal in the decision to focus on epilepsy given the predictivity of the model and increased potential for success in later stage clinical trials (Yuen and Sims, 2014; Gurrell et al., 2019). Despite the negative data with darigabat in generalised anxiety disorder (GAD), potentially due to subtherapeutic exposure being achieved (Simen et al., 2019), the totality of the evidence indicates an important role of the α2/3-subunit containing $GABA_A$ receptors in anxiolysis (see review in Skolnick, 2012). For example, α2/3-selective TPA023 demonstrated anxiolytic potential in GAD (Atack, 2009), highlighting that this mechanism of action (MOA) warrants further investigation in anxiety disorders. Rather than repeat a large and costly clinical trial at higher receptor occupancy (RO) in, for example, GAD patients, an experimental medicine approach was selected to de-risk further development of darigabat in anxiety indications through application of an experimental model of panic. The author is currently the Global Scientific Lead of the darigabat program at Cerevel Therapeutics, responsible for the overall project strategy and execution. The author led the concept and design of the currently ongoing clinical trials and is involved in the execution of each trial.

Figure 5-1 Summary of currently ongoing clinical trials with darigabat

Protocol No./ NCT ID (Status)	Trial Design	Key Objectives	Treatment/Doses	No. of Participant randomisations planned	Key trial design features
CVL-865-SZ-001 / NCT04244175 (Ongoing)	Phase 2, randomised, double blind, placebo-controlled, parallel-group, multi-centre, multiple oral dose trial in patients with drug-resistant focal epilepsy.	To determine the ability of darigabat to reduce the seizures in subjects with drug-resistant focal onset epilepsy.	2-week titration to target maintenance doses of Placebo, 7.5 mg BID, 25 mg BID darigabat for 8 weeks.	150.	Adults (18-75) with a history of 4+ seizures per month for at least 3 months despite treatment with 1-3 antiepileptic drug(s). Primary endpoint: reduction in focal onset seizure frequency collected by electronic diary.
CVL-865-SZ-002 / NCT04686786 (Ongoing)	57-week open label extension, multi-centre, multiple oral dose trial in patients with drug-resistant focal epilepsy (continuation of trial CVL-865-SZ-001 for those patients who wish to participate).	To determine the long-term safety of darigabat in patients with drug-resistant focal epilepsy.	Blinded 2-week titration to target maintenance dose of 25 mg BID darigabat for 52-weeks of unblinded treatment.	Up to 150.	Patients able to join the 57-week open label extension trial after completion of the 8-week maintenance phase of CVL-865-SZ-001

Protocol No./ NCT ID (Status)	Trial Design	Key Objectives	Treatment/Doses	No. of Participant randomisations planned	Key trial design features
CVL-865-HV-001 / NCT04592536 (Ongoing)	Phase 1, randomised, double-blind, placebo- and active-controlled, multiple oral doses, cross-over trial in healthy participants.	To determine the efficacy of darigabat in an experimental medicine model of panic induced by CO_2 inhalation.	4-day titration to target maintenance doses of placebo, 7.5 mg BID, 25 mg BID darigabat, 1mg alprazolam.	54.	Two-way cross-over design to reduce potential habituation effects of repeated CO_2 exposure. Each cohort compared to placebo: Cohort 1 (n=18) - 25 mg BID darigabat. Cohort 2 (n=18) – 1 mg alprazolam (positive control) Cohort 3 (n=18) – 7.5 mg BID darigabat. Primary endpoint: Panic symptoms list-IV.
Abbreviations: BID = twice daily dosing; CO_2 = carbon dioxide; NCT ID = ClinicalTrials.gov Identifier; No. = number. Definition of ongoing clinical trial is a final Clinical Study Report is not available.					

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5.2 Ongoing clinical trials with darigabat

5.2.1 Phase 2 clinical trial in patients with drug-resistant focal epilepsy (CVL-SZ-001; NCT04244175)

Before examining the specific trial design of the Phase 2 trial of darigabat in patients with treatment-resistant epilepsy a brief review of the guiding principles of antiepileptic drug (AED) clinical research is outlined. The European Medicine Agency (EMA) guideline on clinical investigation of medicinal products in the treatment of epileptic disorders is currently under review (see https://www.ema.europa.eu/en/clinical-investigation-medicinal-products-treatment-epileptic-disorders) and the FDA guidelines were last updated in 1981 (see https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-evaluation-antiepileptic-drugs-adults-and-children) but both provide the general guiding principles for new AED research. Drug approvals for AEDs are only granted for the specific seizure types or syndromes investigated in pivotal clinical trials. Therefore, a drug which has demonstrated clinically meaningful efficacy in clinical trials of patients with focal onset seizures will only gain approval for the treatment of patients with focal onset seizures, and separate clinical trials are required in order to be approved for patients with generalised epilepsy, for example. It is therefore important that the seizure type or epilepsy syndrome included is clear and classified correctly using the international classification system set out by, for example, the International League Against Epilepsy in 2017 (Fisher et al., 2017).

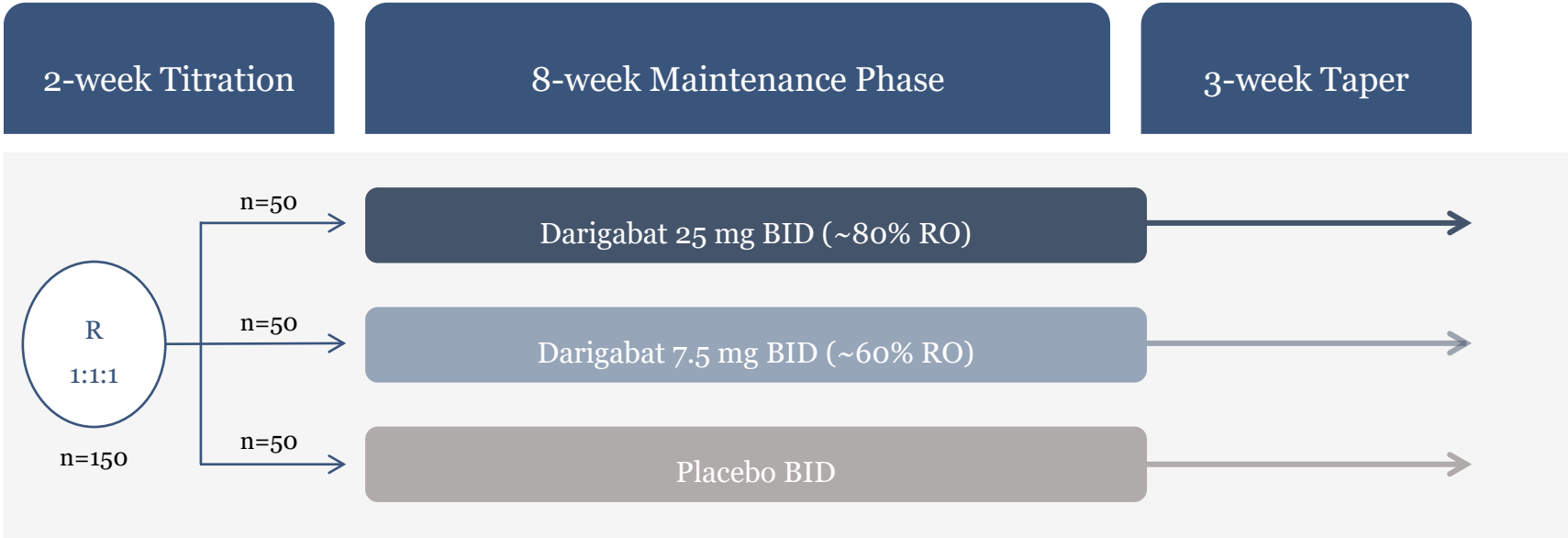
Usually, focal seizure in adults is the first seizure type that is evaluated in clinical development since this is the most commonly occurring seizure type, affecting approximately 60% of epilepsy patients. Furthermore, a substantial proportion (~30%) of patients with focal seizures are not well-controlled and are treatment resistant, highlighting a continued need for innovative research for novel treatments (Kwan et al., 2010). Traditionally, the initial clinical trial evaluation involves assessment of seizure burden in patients who continue to have seizures despite therapy with an adequate regimen of appropriate AEDs and continue to administer their AED therapy. Whilst the background drugs can be confounders of trial execution and interpretation due to, for example, pharmacokinetic (PK) interactions or additive side effects, monotherapy studies

are not considered feasible to identify the potential of a drug in early clinical development. This is due to ethical considerations (i.e., a newly diagnosed patient may achieve seizure freedom from treatment with a currently approved AED), and safety issues associated with placebo-controlled trials with a drug having an unknown efficacy and safety profile.

The regulatory guidelines recommend that pivotal add-on studies should be randomised, double-blind, placebo-controlled parallel group trial design and efficacy should be demonstrated in a minimum of 2 trials. They should include a baseline period in which the number of seizures experienced is captured, a titration period (when applicable), and a maintenance period of ideally 12-weeks during which the target dose is administered. Efficacy endpoints are based on the change in seizure frequency between the treatment maintenance phase and the baseline period excluding the titration period.

The design of the trial with darigabat does not deviate far from the guidelines set out by the regulators. It is a multi-centre, randomised, double-blind, placebo-controlled, parallel-group trial to assess the efficacy, safety, and tolerability of darigabat as adjunctive therapy in adult patients with drug-resistant focal epilepsy (Clinical Trial ID: #NCT04244175, **Figure 5-2**). The proof-of-concept (POC) trial is being conducted in Australia, Spain, and the United States at approximately 60 clinical sites. The trial population includes patients with an appropriate severity level of disease to allow for the detection of anticonvulsant activity with darigabat. The key inclusion criteria include: (a) men and women 18 to 75 years of age with a diagnosis of epilepsy with focal onset, focal aware, focal impaired awareness, or focal to bilateral tonic-clonic seizures for at least two years; (b) drug resistance, defined as lack of disease control despite the use of at least two prior appropriate AEDs (Kwan et al., 2010); (c) treatment with at least one but no more than three AEDs and; (d) a history of an average of four or more spontaneous and observable seizures per 28-day period for at least three months.

Figure 5-2 Overview of design of Phase 2 clinical trial in patients with drug-resistant focal epilepsy (CVL-SZ-001; NCT04244175)



Abbreviations: AED = antiepileptic drug; BID = twice daily; R = randomisation; RO = receptor occupancy.

Schematic of the double-blind, randomised, placebo-controlled Phase 2 clinical trial in patients with drug-resistant focal epilepsy (NCT04244175). The trial is a parallel group design comparing darigabat at 25 mg BID and 7.5 mg BID with placebo and comprises of a 2-week titration period following by an 8-week maintenance period and a 3-week taper period. Patients who complete the 8-week maintenance phase of the trial are eligible to join the 57-week open label extension trial.

After an 8-week screening period eligible patients will enter a 10- or 13-week treatment period, which includes (1) a two-week titration phase, which was designed with the knowledge from prior clinical trials that some of the early somnolence side effects of darigabat may be mitigated by titration, (2) an eight-week maintenance phase, and (3) either a three-week taper period or enrollment into a 57-week open-label extension (OLE) trial (**Figure 5-2**). The 8-week maintenance phase is shorter than the 12-weeks recommended in the guidelines primarily because >13-week nonclinical toxicity data was unavailable at the time the trial was designed. Although the longer-term nonclinical toxicity data became available prior the trial being initiated the length of the maintenance was not changed due to the significant increase in cost. Furthermore, cenobamate was recently approved by the FDA for use in the same patient population with a “supportive to pivotal” Phase 2 trial with a short 6-week maintenance period followed by a single pivotal 12-week Phase 3 trial (Chung et al., 2020). Therefore, it is precedented that a regulatory agency has previously accepted shorter trials as part of the New Drug Approval package. The three-week taper phase is designed to mitigate possible risks of rebound seizures from too-rapid withdrawal from darigabat, common practice amongst all AEDs.

150 eligible patients who have experienced at least eight focal onset seizures during the 8-week screening period will be randomised 1:1:1 to one of the following three arms: 25 mg BID of darigabat; 7.5 mg BID of darigabat, or placebo BID (**Figure 5-2**). The two doses of darigabat have been selected based on the safety and tolerability data from previous Phase 1 trials, and the RO modeling based on positron emission tomography (PET) characterisation (Nickolls et al., 2018). Furthermore, the two doses chosen for the focal epilepsy trial achieve similar systemic drug concentrations to the two doses that demonstrated efficacy in the Phase 2 proof-of-principle photosensitive epilepsy (Gurrell et al., 2019), a model which has been shown not only to predict efficacy in patients with epilepsy, but also the minimum effective dose (Yuen and Sims, 2014).

Throughout the screening period and over the course of the trial, patients will use an electronic seizure diary to capture their seizure events. The primary endpoint to evaluate the efficacy of darigabat will be the reduction in frequency of focal onset seizures during the maintenance phase versus baseline as compared to the placebo group. This will be

calculated as Rratio=(T—B)/(T+B) ×100, where T represents the seizure frequency rate per week in the maintenance phase and B represents the seizure frequency rate per week in the baseline screening period. The Rratio is between -100 and 100, where negative values will indicate reduction in seizure rate and positive values indicate increase in seizure rate during treatment. Reduction in seizure frequency using Rratio has been used as the primary endpoint in prior registrational trials of drugs for adjunctive treatment of focal epilepsy, including for gabapentin and pregabalin (Mohanraj and Brodie, 2003). A sample size of 50 participants in each group was selected to provide at least 80% power to detect a difference in the means of the primary outcome measure (Rratio) of -20, which is approximately equivalent to a 33% reduction in seizures. Therefore, this trial is sufficiently powered to detect effect sizes like those observed with other AEDs like levetiracetam (Keppra) in the clinical trial setting.

Key secondary efficacy endpoints will include responder rate, defined as the percent of patients who experience at least a 50% reduction in focal onset seizure frequency compared to baseline, and seizure frequency per week over the 8-week maintenance phase. Change in seizure frequency over time may provide some insight into maintenance of efficacy, however, 8-weeks is a relatively short timeframe in which to do so. Previous literature has demonstrated that tolerance to benzodiazepines (BZDs) may develop anytime from after a few days of treatment (Hambert et al., 1970) to 18-months of long-term therapy (Hanson and Menkes, 1972), with the majority of reports indicating that tolerance developed within 1 to 6 months of continuous BZD therapy (Gidal et al., 2016). However, data from the OLE clinical trial in which darigabat is administered for 57-weeks has the potential to address the issue of tolerance (see **Section 5.2.1**). Safety parameters will include assessment of withdrawal symptoms during the taper phase of the trial using a modified version of the Clinical Institute Withdrawal Assessment Scale – Benzodiazepines (mCIWA-B). The mCIWA-B is a sensitive instrument to measure withdrawal under conditions where there is a taper of medication (rather than abrupt discontinuation) (Busto et al., 1989) and was modified to remove the in-person assessment of patients so the scale may be delivered by telephone. Whilst this deviates from the standard approach, it does reduce the burden of visits to the clinical research

site which is beneficial to the patients. The data will be used to indicate whether this drug has a lower propensity to induce withdrawal symptoms compared to data reported with BZDs.

The trial was initiated in the second half of 2020 after a delay due to the Covid-19 pandemic and is expected to complete in the second half 2022. The totality of the activity and tolerability data that will be generated in this trial will guide further clinical development of darigabat in epilepsy, and potentially of other molecules currently being developed with a similar MOA.

5.2.2 Open label extension trial in patients with drug-resistant focal epilepsy (CVL-SZ-002; NCT04686786)

Long-term data in epilepsy clinical trials is generated by continuation of add-on studies or by conducting OLE studies to assess absence of tolerance and maintenance of safety. Treatment retention rate is recommended as a global indicator of clinical effectiveness and a one-year extension trial duration is considered the minimum period per EMA guidelines. For darigabat, data from the OLE trial will also provide some information of the longevity of any efficacy observed given the extended administration period. Initiating an open-label trial at the end of Phase 2 is not a requirement as they can be recruited to follow-on Phase 3 clinical trial instead, however they are a recruitment incentive into the placebo-controlled and randomised Phase 2/3 studies. Personal communication at meetings with key opinion leaders advised that without the ability for the patient to continue to receive ongoing investigative treatment after the completion of the randomised trial, many patients, or indeed clinical research sites, would not participate in the trial. The disadvantage to executing an OLE prior to knowledge of efficacy data from an earlier trial is the substantial cost of the trials, which still require significant oversight for what is likely to be >2 years. However, the OLE numbers do contribute to the overall number of subjects that are required to complete long-term safety assessments before approval of a drug is granted.

Here, subjects are permitted to roll-over into the OLE once they have completed the 8-week maintenance phase of the Phase 2 POC trial (Clinical Trial Identifier #NCT04686786). They do it at that point to avoid the taper phase of the POC and it is

designed in such a way that the titration into the OLE is blinded so that the last treatment received in the POC is not unintentionally revealed. At the time of writing several patients had completed the maintenance period of the OLE and had opted to join the OLE.

Data from both the POC and OLE have the potential to inform future clinical trial design for a Phase 3 trial of darigabat in drug-resistant focal epilepsy, and if the drug is safe and well tolerated then there is a potential that the Phase 3 trial design and associated OLE may have a reduced safety monitoring requirement, if agreed by regulatory authorities.

5.2.3 Phase 1 experimental medicine model of panic in healthy participants (CVL-HV-001; NCT04592536)

The Phase 2 trial with darigabat in patients with GAD potentially did not achieve sufficient RO levels to demonstrate anxiolysis and as such, the anxiolytic potential of darigabat is yet to be fully evaluated (see **Chapter 3.3.3**; Simen et al., 2019). Given the significant cost, resource, and time to "repeat" the GAD trial at higher doses, an alternative model was sought to establish preliminary evidence of anxiolytic activity and to be decision-making as to whether to advance to a larger, more costly trial.

A range of models have been used to establish evidence of legitimacy for novel treatment development in anxiety disorders, including lactate infusion and cholecystokinin challenge in panic disorder, the Trier social stress test in social anxiety disorder, oxytocin administration in separation anxiety disorder, and carbon dioxide inhalation in panic disorder and GAD (Bailey et al., 2011). The latter was selected as it is a commonly used experimental approach in the investigation of induced anxiety/panic, including recently with JNJ-61393215, a selective orexin-1 antagonist, which subsequently advanced to a Phase 2 trial in patients with major depressive disorder with anxious distress (Salvadore et al., 2020; Clinical Trials identifier #NCT04080752).

Brief inhalation of air with higher-than-normal concentrations of CO_2 (commonly 35% CO_2) is associated with a robust fear response as measured by behaviour and panic ratings in both healthy participants and patients with panic disorder (Bailey et al., 2011). Whilst inhalation of a lower concentration of CO_2 (typically ~ 7.5%) over a 20-minute period can elicit the characteristics of generalised anxiety, due to the recent precedented

use of the model in “panic mode” using the BZD alprazolam as a positive control (Salvadore et al., 2020), brief inhalation of 35% CO_2 was selected to investigate the effects of darigabat in the model. Furthermore, alprazolam is approved for the treatment of panic disorder.

Similarities exist between experiencing experimentally-induced hypercapnia in healthy participants and in the panic symptoms experienced by panic disorder patients. These include exhibiting a marked fear response associated with physiological increases in blood pressure, an adaptive decrease in heart rate, increases in fear and panic as measured by visual analogue scales (VAS) (e.g., VAS Fear), and the Panic Symptom List (PSL) which utilises questions based on anxiety and panic Diagnostic and Statistical Manual of Mental Disorders (DSM) categories (PSL: **Table 5-1**). For the VAS Fear, feelings of fear are rated by subjects using a VAS, consisting of a horizontal line 100 mm in length. Subjects indicate their fear level along the line, with 0 corresponding to “no fear” and 100 corresponding to “the most fear possible.” The VAS provides a good estimate of rapid changes of aspects of mood states. Individual responses to CO_2 inhalation vary, with some individuals being more sensitive than others, and patients with panic disorder being at the highest end of the sensitivity spectrum (Leibold et al., 2016). In terms of predictive validity (the degree to which the model predicts outcome in patients), a quantitative comparison of physiological outcome measurements in healthy participants have been shown to be comparable to those of panic disorder patients (Bailey et al., 2011). In terms of the construct validity, referring to the degree of etiological similarities underlaying the behavioural response in the model and the disorder, drugs that are approved for the treatment of anxiety/panic reduce panic symptoms elicited by CO_2 exposure in healthy participants, including BZDs, selective serotonin reuptake inhibitors, and selective noradrenaline reuptake inhibitors (Bailey et al., 2011).

Table 5-1 Panic Symptom List-IV (PSL-IV) individual items

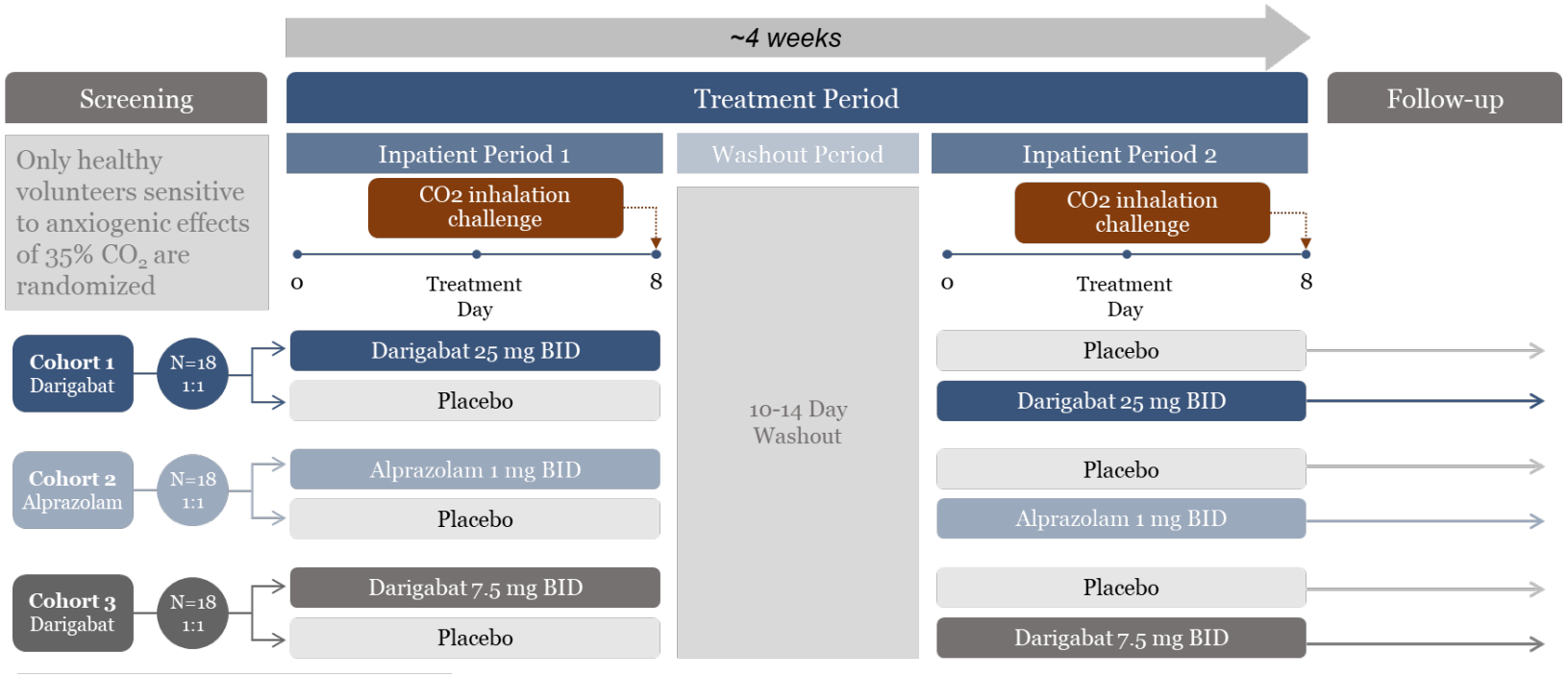
Symptom
Dizziness
Feeling of choking/gasping for breath
Hot flashes/cold shiver
Nausea
Palpitations
Sweating
Shortness of breath
Numb/tingling
Depersonalisation/derealisation
Fear of dying
Fear of losing control
Chest pain/discomfort
Trembling/shaking
The PSL-IV consists of a questionnaire containing 13 items, where each item is derived from those listed for panic disorder in the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-4). The PSL-IV uses an ordinal scale, ranging from 0 (not at all) to 4 (very severe). The PSL-IV total score will be derived as the sum of scores of the 13 items (with a maximum of score 52).

More recently, the novel orexin-1 receptor antagonist JNJ-61393215 and the positive control BZD alprazolam induced a statistically significant reduction in anxiety/panic symptoms induced by inhalation of 35% CO_2 in healthy participants according to the

primary outcome measure the Panic Symptom List-IV (PSL-IV) (Salvadore et al., 2020; Clinical Trials identifier #NCT04080752). A similar clinical trial design was developed to investigate the anxiolytic potential of darigabat. The trial is a randomised, double-blind, placebo- and active-controlled trial designed to assess the anxiolytic efficacy of multiple doses of darigabat administered over 8-days on symptoms of acute anxiety or panic evoked by the CO_2 double-breath inhalation challenge in healthy participants. The trial is a two-period, two-sequence crossover design with 3 cohorts comparing darigabat 25 mg BID, darigabat 7.5 mg BID, and alprazolam 1 mg BID to placebo (**Figure 5-3**). The trial includes a 3-week screening period, 2 in-clinic treatment periods of 9 days each, 10 to 14 days washout between periods, and a 30-day safety follow up period. During each inpatient period, there will be a 4-day titration phase followed by a 4-day maintenance phase. Only individuals who are found to be sensitive to the anxiogenic effects of 35% CO_2 double-breath inhalation at screening will be eligible for randomisation into the treatment period. Sensitivity to the anxiogenic effects on this protocol is defined as an increase from pre-CO_2 to post-CO_2 challenge in the following: PSL-IV score ≥ 4 with at least 1-point increase for at least 4 of the symptoms specified in the PSL-IV and an increase on the VAS Fear of ≥ 25 mm.

The high dose of 25 mg BID is designed to fully test the therapeutic potential of darigabat as treatment of anxiogenic symptoms induced by the CO_2 challenge. This dose is higher than that tested in the GAD trial in which a lack of efficacy was observed (Simen et al., 2019) and achieves an exposure level of darigabat comparable to that at which the peak pharmacodynamic (PD) effects in saccadic peak velocity (SPV), a reliable biomarker of α2/3 activity, and approximately 80% RO (Nickolls et al., 2018). The low dose of 7.5 mg BID is anticipated to have a physiologically significant but submaximal effect (based on SPV) with RO at approximately 60%. The lower dose is intended to provide sufficient data to fully understand the relationship between exposures and clinical endpoints to facilitate rational dose selection in future clinical trials, and aid interpretation of the GAD trial in which both 2.5 and 7.5 mg BID doses of darigabat was not anxiolytic (Simen et al., 2019). The active control is alprazolam 1 mg BID, a BZD approved for use in panic disorder,

Figure 5-3 Overview of design of Phase 1 clinical trial of darigabat in CO_2 challenge model of acute panic in healthy participants (CVL-865-HV-001; NCT04592536)



Abbreviations: BID = twice daily; N = number

Schematic of the randomised, double-blind, placebo- and active-controlled Phase 1 clinical trial to assess the anxiolytic efficacy of multiple doses of darigabat on symptoms of acute anxiety/panic evoked by the CO_2 double-breath inhalation challenge in healthy participants. The trial is a 2-period, 2-sequence crossover design with 3 cohorts comparing darigabat at 25 mg BID and 7.5 mg BID, alprazolam 1 mg BID with placebo. The trial includes a 3-week screening period, 2 in-clinic treatment periods of 9 days each, 10 to 14 days washout between periods, and a 30-day safety follow up period. During each inpatient period, there will be a 4-day titration phase followed by a 4-day maintenance phase.

which will be used to demonstrate assay sensitivity and to facilitate interpretation of the PD effects of darigabat.

The primary outcome measure is change in the PSL-IV and secondary endpoints include change in the VAS Fear and PD assessments using the NeuroCart battery of tests. The trial plans to enrol 18 participants per cohort (54 participants in total), the sample size being powered to detect an effect size like that observed with alprazolam previously (Salvadore et al., 2020). Although it is challenging to quantitatively define decision criteria related to the efficacy outcomes in the trial, darigabat will be compared to both placebo and alprazolam to determine the anxiolytic potential of darigabat when the data is available.

At the time of writing, the trial has been initiated, with full results expected in the second half of 2021. The data from this hypercapnia trial will inform the future development potential of darigabat in anxiety disorder.

5.3 Future direction

Positive data in the Phase 2 trial in patients with drug-resistant focal seizures will potentially result in initiation of Phase 3 clinical trials in support of a New Drug Approval registration in focal epilepsy. However, given the broad-spectrum effectiveness of darigabat (and BZDs) in animal models of focal and generalised epilepsies, positive data in the focal epilepsy trial could also pave the way for clinical trials in other seizure types, such as generalised, or in rare epilepsies.

If there is evidence of anxiolytic activity in the Phase 1 experimental panic model further trials to investigate the therapeutic potential in patients with an anxiety disorder may be warranted. Anxiety disorders are a complex and diverse category of psychiatric diseases affecting hundreds of millions of patients worldwide. There are several anxiety disorders specified in the DSM-5 including GAD, panic disorder, social anxiety disorder, specific phobias, post-traumatic stress disorder, and obsessive-compulsive disorder. Given that BZDs are not labelled for use in post-traumatic stress disorder, specific phobias and obsessive-compulsive disorder, and the reported lack of evidence of the effectiveness of BZDs in these indications potentially due in part to the development of tolerance

(Hollander et al., 2003; Guina et al., 2015), the future development of darigabat will potentially focus on one or more of GAD, panic disorder, or social anxiety disorder. The exact patient population will be determined based on the readout of data from the ongoing hypercapnia trial and an assessment of unmet need and commercial evaluation of each anxiety indication. Positive data in the hypercapnia challenge model could also increase the interest in psychiatric indications outside of anxiety and panic disorders, for example in agitation associated with Alzheimer's disease where BZDs are used but at the risk of increasing cognitive impairment or motor impairment which is associated with falls.

5.4 Discussion

The current trials are designed to explore a number of key questions about the hypothesised potential of subtype-selective $GABA_A$ receptor PAMs in the clinic. They are devised to reveal the potential of darigabat to treat epilepsy and anxiety disorders and provide key safety information related to long-term administration at what are believed to be therapeutic doses. The focal epilepsy trials are an opportunity to decipher whether it is devoid of efficacy tolerance and withdrawal symptoms on discontinuation of dosing, both of which are major issues associated with BZD use. The hypercapnia trial has the potential to answer a yet unanswered question related to the therapeutic promise of darigabat to treat anxiety disorders and to further understand the levels of RO required for efficacy, although patient studies will be needed to confirm the translatability of the model. The data from these trials will further our understanding of the complex $GABA_A$ receptor and neurological conditions such as epilepsy and anxiety disorder, which in turn has the potential to positively impact patients and families living with these disorders by advancing the scientific understanding of these conditions and their susceptibility to treatment through various approaches.

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6 CONCLUSION

The search for new drugs to treat patients with neurological and neuropsychiatric disorders is long and challenging. The targets have become less tractable, the clinical trials more complicated, and the hurdles higher. However, across the timeframe that this review spans, which includes learnings from more than a decade, the toolkit with which to conduct research and development has expanded and an emphasis has emerged with regard to translational strategies to aid decision-making. Pharmacodynamic (PD) biomarkers now have a well-established role in both nonclinical and early clinical development in many pharmaceutical companies (Morgan et al., 2012; Cook et al., 2014; Dolgos et al., 2016). This early investment in programs to develop clinical biomarkers and the utilisation of translational sciences to underpin the design and progression of molecules capable of testing the mechanism in patients has begun to reap rewards and has been attributed in part to an increase in successful outcomes in Phase 2 (Wu et al., 2021).

The darigabat program leveraged this new organisational momentum and a biomarker-based strategy has been embedded throughout the program to answer key research questions. First, strategies have been utilised to enable translation of the nonclinical pharmacology profile into a clinical profile in a disease-agnostic way, with this approach successful in establishing increases in quantitative electroencephalogram (EEG) β frequency as a translatable biomarker for the α2-subtype selective target modulation (Nickolls et al., 2018). Second, the measurement of mechanistically relevant biomarkers in the first-in-human (FIH) trial was essential in establishing that the pharmacology of darigabat is differentiated from a benzodiazepine (BZD), a critical differential characteristic for the continued development of a novel drug versus available generic treatments. This approach provided confirmation that darigabat modulates the desired α2/3-mediated pharmacology more robustly than a BZD and that the undesired α1/5-mediated effects are minimal in comparison. Furthermore, the biomarkers demonstrate that the overall pharmacokinetic/pharmacodynamic profile of darigabat in healthy participants correlates with both the nonclinical binding and functional selectivity profile (Nickolls et al., 2018).

Of note, the dose-dependent modulation of saccadic peak velocity (SPV) by darigabat is closely correlated to the receptor occupancy (RO) and this biomarker data was used to select doses for both the single and multiple dose trials. Although it is clear that the biomarkers utilised in the FIH in healthy participants are not clinical outcome measures, SPV was reportedly predictive of clinical efficacy for anxiety (de Visser et al., 2003). Accordingly, darigabat was predicted to demonstrate anxiolytic efficacy at relatively low (< 50%) RO and clinical trials in the difficult to treat patient populations of anxiety (and pain) were conducted. Disappointingly, both trials failed to demonstrate a therapeutic benefit of the drug (Gurrell et al., 2018; Simen et al., 2019). At the time of these trials biomarker data was already available to indicate that the previously selected doses were submaximal, although at that time were not permissible for progression due to a self-imposed multiple dose trial maximum exposure limitation. Critically, at that time, the removal of the self-imposed limitation on dose was perhaps perceived as a higher hurdle compared to achieving efficacy in these patients at a lower dose as predicated by the SPV results and nonclinical efficacy data. When a multiple ascending dose trial was conducted in healthy participants with permission from the United States Food and Drug Administration at doses exceeding the self-imposed dose cap it confirmed the generally safe and tolerable profile of darigabat at > 80% RO (Gurrell et al., 2021). In hindsight, the decision to execute clinical trials in difficult to treat patient populations at doses that do not maximally modulate the receptor was flawed and it currently remains unclear whether darigabat will provide clinical benefit in these patient populations. Furthermore, data from the single dose PainCart trial with darigabat demonstrated that there were more modest effects of darigabat at approximately 50% versus 80% RO, and compared to pregabalin (van Amerongen et al., 2019). It is tempting to believe that if this data had been available prior to the chronic low back pain (CLBP) trial that the emphasis may have shifted to taking the appropriate steps to remove the dose cap and permit an increase in dose to achieve > 50% RO rather than move forward with the patient studies with the doses that achieve less than maximal PD activity.

There was, however, success in the single dose photosensitive epilepsy trial (Gurrell et al., 2019) which, although using an “efficacy” outcome measure, is perhaps on the border

of being considered a biomarker. The robustness of this data and the apparent predictivity of the model to anticonvulsant efficacy in wide epilepsy populations (Yuen and Sims, 2014) was viewed as providing sufficient confidence to warrant further clinical trials in epilepsy patients and directly resulted in this program continuing into later stage clinical development. To our knowledge, this was the first clinical trial to be conducted with an α2/3/5-subtype selective $GABA_A$ receptor positive allosteric modulator (PAM) in patients with epilepsy. Importantly, the data from this trial and from studies with darigabat in nonclinical models of seizures (Duveau et al., 2019; Owen et al., 2019; Bialer et al., 2020) suggest that the α2 subunit is important in conferring the anticonvulsant activity of BZDs at $GABA_A$ receptors, although activity at α1 subunits is expected to be negligible a potential contribution cannot be ruled out. The current proof-of-concept and 57-week open label extension trials with darigabat in patients with drug-resistant focal epilepsy (NCT04244175; NCT04686786) will provide more definitive data in clarifying if the concept that a subtype-selective $GABA_A$ receptor PAM with minimal activity at α1-containing receptors will have utility in patients with seizures.

Both successes and failures continue to provide invaluable information that help direct science to further progress. The earlier failures with non-selective but lower functional efficacy PAMs such as bretazenil coupled with the advent of new molecular cloning techniques attributing specific pharmacology of BZDs to specific subunit subtypes helped refine the hypothesis and resulted in program strategies targeting both lower functional efficacy and α subunit selectivity as exemplified by the darigabat program. Although we still have an incomplete understanding of the role of subtype-selective $GABA_A$ receptor PAMs in a range of neurological and neuropsychiatric disorders, the biomarker strategy has served multiple purposes here. It has furthered understanding of the relationship between RO and PD (and efficacy), with the emphasis focused on achieving high (>50%) RO with compounds with a relatively modest level of functional activity compared to a BZD. It has aided interpretation of past trials in pain and anxiety and underpinned the importance of α2 subunits in epilepsy following the robust efficacy of darigabat in the photoepilepsy model. More work is required to understand the differentiation between $GABA_A$ receptor subtype-selective PAMs and BZDs with respect to efficacy and in terms

of abuse liability and tolerance. The currently ongoing trials in epilepsy and panic will go some way to answer key questions about the therapeutic potential of this targeted therapeutic approach, which in turn has the potential to positively impact patients and families living with these disorders.

When she joined a small exploratory team in 2007 tasked with identifying subtype-selective $GABA_A$ receptor PAMs to move through the research and development process, the author never anticipated that this work would continue over the next 15 or so years. The time was right to acquire new skills as the area of biomarker research grew and the author had the opportunity, desire, skills, and support from those around her to make a viable contribution to the field of $GABA_A$ receptor pharmacology. The next chapters of this program are yet to evolve with critical readouts from clinical trials expected over the next years at which point a new era in $GABA_A$ receptor pharmacology may well be revealed.

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Appendix 1. Publications which contribute to this PhD by publication

Publications are listed in chronological order.

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