ABSTRACT
The muscarinic acetylcholine M1 receptor protein is known to play a role in hippocampal-based and short-term memory. It has been shown to improve cognition and modify Alzheimer’s disease (AD) properties in animal models. As a result, muscarinic M1 agonism has been identified as a target for AD treatment.

Initial drug development programs targeting the muscarinic M1 receptor were curtailed by unacceptable adverse events due to a lack of specificity for the M1 subtype. More recent research has focused on allosteric agonists which have greater M1 receptor specificity. As these compounds start to move into clinical development, there is a need for biomarkers of M1 agonism to help speed up their development.

INTRODUCTION TO SOLUTIONS
Using Thomson Reuters Integrity and MetaCore, we investigate known markers of muscarinic M1 agonism, identify potential new biomarkers and assess their suitability for further validation and use in clinical trials.

METACORE
MetaCore is an integrated knowledge database and software suite for pathway analysis of experimental data and gene lists. The scope of data types includes microarray and sequence-based gene expression, SNPs and CGH arrays, proteomics, metabolomics, Co-IP pull-out, and other custom interactions.

MetaCore is based on a proprietary manually-curated database of human protein–protein, protein-DNA, and protein-compound interactions, metabolic and signaling pathways for human, mouse, and rat, supported by proprietary ontologies and controlled vocabulary. The analytical package includes easy to use, intuitive tools for searching, data visualization, mapping and exchange, building biological networks, and interactome analysis.

THOMSON REUTERS INTEGRITY WITH THE BIOMARKERS MODULE
Integrity is a unique drug discovery database that integrates biological, chemical, and pharmacological data to support better informed decision-making. It is built, populated, and updated by scientists who bring the latest information, expert analysis, and current opinion on potential and precedent targets, and translational research, including biomarkers.

The Biomarkers Module of Integrity provides high-quality, continuously updated information supporting biomarker research at every stage of drug research and development. It is the first biomarker database to provide standardized terminology and to classify biomarkers reliably into lifecycle phases and disciplines to help identify the right biomarker with the right utility at the right time.

BACKGROUND
Alzheimer’s disease is a progressive and ultimately fatal brain degenerative disorder. It is characterized by formation of amyloid plaques and neurofibrillary tangles in the brain and a loss of basal forebrain cholinergic neurons. As a result, patients exhibit memory and cognitive deficits.

In 2006, more than 26 million people worldwide were affected by AD. By 2050, 1 in 85 people is expected to be living with Alzheimer’s disease and 43 percent of those affected are expected to need a high level of care (1).

Given the loss of cholinergic neurons associated with AD, efforts to treat the disease have been focused on compounds that increase cholinergic activity in the brain. Of the six drugs so far launched to treat AD, four are cholinesterase inhibitors (tacrine hydrochloride, galantamine hydrobromide, donepezil hydrochloride and rivastigmine tartrate), with rivastigmine tartrate also being a butrylcholinesterase inhibitor (Source: Integrity). Other approaches have focused on compounds which act as agonists of nicotinic or muscarinic acetylcholine receptors (2, 3).

Five muscarinic acetylcholine receptor subtypes have been cloned. All five subtypes are G-protein coupled receptors that play a role in neurotransmission. The receptors are expressed in different tissues throughout the CNS with the M1 receptor subtype being primarily expressed in the cortex and hippocampus, the regions of the brain most closely associated with memory and cognition.
The muscarinic acetylcholine M1 receptor protein (ACM1) is known to play a role in hippocampal-based and short-term memory (4). It has been shown to improve cognition and modify Alzheimer’s disease properties in animal models (5, 6). As a result, muscarinic M1 agonism has been investigated as a target for AD treatment.

Clinical utility of early M1 agonists in AD patients was limited by low efficacy and unacceptable side effects such as diarrhea, bradycardia, sweating and salivation (3). The cause was identified as a lack of selectivity for the M1 receptor subtype. M1 agonists also interacted with muscarinic M3 receptor. Indeed, the only M1 agonist to be marketed, Cevimeline hydrochloride, is a joint M1/M3 agonist approved in the USA and Japan for treatment of dry mouth in patients with Sjogren’s disease (Source: Integrity).

These “first generation” M1 agonists all targeted the orthosteric acetylcholine binding site on the receptor. The orthosteric site is highly conserved between the M1, M3 and M5 receptor subtypes, thus explaining the poor selectivity of the drugs and resultant increase in toxicity.

In 2002, Spalding et al identified agonists of M1, M3 and M5 which acted at allosteric binding sites removed from the conserved orthosteric site (7). These findings helped to reinvigorate the search for effective muscarinic M1 agonists (8). Currently there are several allosteric M1 agonists and positive allosteric M1 modulators at the preclinical phase of development, with 29 of these being investigated for treating AD. Other conditions under investigation are schizophrenia and related psychotic disorders (see Figure 2; source: Integrity).
MATERIAL & METHODS

Using the Biomarkers Module of Integrity, known markers of response to M1 agonists were explored. Then potential downstream biomarkers of M1 agonism were retrieved in MetaCore and visualized using the "expand-by-one" network-building algorithm. This process identified beta secretase (BACE1) enzyme activity as a possible biomarker of M1 agonism. The feasibility of using BACE1 enzyme levels as a biomarker was investigated further using the Biomarkers Module.

RESULTS & DISCUSSION

As a first step in identifying biomarkers of muscarinic M1 treatment response, the Biomarkers Module was used to investigate whether or not there were any reports of biomarkers which correlated with response to muscarinic M1 agonists. A search of the Biomarkers Module highlighted a total of 10 biomarkers, four of which were from Alzheimer’s disease. Further investigation in the Biomarkers Module showed that the four biomarkers were from the same preclinical study reported at the Society for Neuroscience Annual Meeting in 2012 (9).

Having found that no biomarkers of muscarinic M1 receptor agonism have yet been reported to work in a clinical setting, it was decided to use MetaCore to investigate downstream effectors of ACM1. In MetaCore, we learned that a total of 915 direct and indirect interactions involving ACM1 had been reported in the scientific literature and of these, 25 were downstream of ACM1. We hypothesized that because the 25 biological components are affected by changes to ACM1, they represent potential biomarkers of ACM1 agonism.

To increase the likelihood of finding a biomarker which is amenable to measurement, we decided to prioritize downstream objects which interact directly with ACM1 and which are localized to the cell membrane. In order to visualize the subcellular localization of potential biomarkers, we utilized the "expand-by-one" algorithm in MetaCore to build a network for ACM1 and all its high-trust, downstream interactions (see Figure 3). Three hypothetical biomarkers were identified that met these criteria as follows: short transient receptor potential channel 6 (TRPC6), G protein-activated inward rectifier potassium channel 1 (Kir3.1) and the membrane-bound enzyme beta-secretase (BACE1).
In the light of toxicity issues encountered by “first generation,” non-selective muscarinic M1 agonists, one essential criterion for our biomarkers is that they should be specific for M1 agonism and not be affected by agonism of any of the other muscarinic receptor subtypes. To investigate whether or not the hypothetical biomarkers were also known to interact with muscarinic M3 receptor (ACM3), we built a second network utilizing the MetaCore “expand-by-one” algorithm (see Figure 4A). From the resultant network we saw that TRPC6 and Kir3.1 interact downstream of both ACM1 and ACM3 making them far less attractive as biomarkers for clinical studies of M1 agonists. The network clearly showed that BACE1 was the only one of our potential biomarkers that is specific for ACM1. The red arrow on the network from ACM1 to BACE1 indicates that ACM1 inhibits BACE1 (10), whereas green arrows on the network indicate activation. We therefore concluded that that agonism of muscarinic M1 receptor is likely to result in a decrease in BACE1 activity.

FIGURE 3: Network showing subcellular localization of potential biomarkers immediately downstream of muscarinic M1 receptor. Subcellular localization of all high-trust, downstream interactions of ACM1 was visualized using the “expand-by-one” network-building algorithm in MetaCore. Orange circles indicate potential biomarkers localized in cell membrane which are not components of a group or complex.
Knowing that BACE1 enzyme activity is likely to be reduced by changes in ACM1 caused by the action of M1 agonists or positive allosteric modulators, our next step was to investigate whether a decrease in BACE1 activity could be linked to Alzheimer’s disease at a mechanistic level. If so, it would imply that changes in the biomarker also have an impact on AD and therefore represent a good surrogate for the therapeutic effect of ACM1 receptor agonism.

By overlaying causal associations related to Alzheimer’s disease (highlighted by blue circles in Figure 4B), it was confirmed that BACE1 is causally associated with AD. The enzyme is known to play a role in amyloidogenesis, the process by which beta amyloid (A-beta) is generated (11). Deposition of A-beta in the brain forms the characteristic amyloid plaques found in AD sufferers. Thus we concluded that a probable mechanism by which muscarinic M1 receptor agonists exert their therapeutic effect in AD is by inhibiting enzyme activity of BACE1 and thereby reducing formation of A-beta.

Having hypothesized that BACE1 enzyme activity could be used as a biomarker of M1 agonism, we searched the Biomarkers Module to ascertain whether or not BACE1 activity has already been used as a biomarker in AD patients. If so, this would validate our hypothesis as well as providing information on suitable assay methods. We found a total of 12 reported uses. Of these, the most commonly used sample type was cerebrospinal fluid (CSF).
Using CSF requires a lumbar puncture which is a difficult and expensive procedure for healthcare professionals and highly invasive for patients. Given that BACE1 is found in most tissues of the body, not only in the brain (12), we chose to focus on the small number of studies to date that have investigated the feasibility of measuring levels of BACE1 in blood tissues. From one recent study, we learned that AD patients showed a significant increase in serum BACE1 enzyme activity (p < 0.005) compared to age-matched, non-demented controls (13). The fact that BACE1 activity is increased in the serum of patients with Alzheimer’s disease further supports our conclusion that a decrease in BACE1 activity would be a suitable biomarker for muscarinic M1 receptor agonism as well as being therapeutically beneficial to the patient.

CONCLUSION

We used Thomson Reuters Integrity with the Biomarkers Module and MetaCore to investigate known markers of muscarinic M1 agonism, identify potential new biomarkers and assess their suitability for further validation and use in clinical trials. We found that:

- Agonism of the muscarinic M1 receptor is likely to result in a decrease in activity of the beta secretase enzyme (BACE1)
- BACE1 interacts directly downstream of the muscarinic M1 receptor but not of the muscarinic M3 receptor, thus making BACE1 a candidate biomarker for selective M1 agonism
- A probable mechanism by which muscarinic M1 receptor agonists exert their therapeutic effect in Alzheimer’s disease is by inhibiting BACE1 activity and thereby reducing formation of A-beta
- Recent studies indicate that measuring BACE1 activity in serum is feasible

We conclude that a decrease in BACE1 enzyme activity in serum warrants further experimental investigation as a biomarker of muscarinic M1 agonism in AD patients.

SUGGESTED NEXT STEPS

- Build further networks in MetaCore with ACM1 and muscarinic M2, M4 or M5 receptors to confirm that BACE1 is selective for the M1 receptor subtype only
- Set up an alert in the Biomarkers Module of Integrity to be notified when any biomarkers from clinical studies of M1 agonists enter the database
- Establish BACE1 baseline activity levels in serum and validate experimentally that BACE1 enzyme activity levels are decreased in response to M1 agonism
REFERENCES
