BACE2 as a new diabetes target: a patent review (2010 – 2012)

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Introduction: When two novel aspartyl proteases were published in 1999 and 2000, beta-site APP-cleaving enzyme 1 (BACE1) was confirmed as the long sought after beta-secretase and Alzheimer’s disease drug target. However, the role of its parologue, BACE2, proved elusive until a 2011 publication implicated it as a Collectrin (TMEM27) secretase controlling pancreatic beta-cell proliferation and a new therapeutic intervention for diabetes.

Areas covered: This review, using SureChemOpen, encompasses early validation compounds and small-molecule BACE2 inhibitors for diabetes. Since 2010, one assay patent and several chemical series have been published by Roche but these were followed by filings from Novartis and Schering in 2012. The patents from these three companies include BACE2-only filings but also some specifying both BACE1 and BACE2 inhibitors.

Expert opinion: Roche’s early collaborative target validation has given them a lead in BACE2 medicinal chemistry. However, the extensive data output for BACE1 in patents and papers over the last decade, plus liganded crystal structures for both proteases, should expedite the design of BACE2 inhibitors by other organisations. This may also shorten the development time for clinical candidates that, unlike those now entering Phase I trials for BACE1, would not need to be brain-penetrant.

Keywords: BACE1, BACE2, diabetes, IC50s, inhibitors, TMEM27

1. Introduction

Diabetes mellitus type II (T2DM) is one of the major diseases of the developed world with healthcare costs in the hundreds of billions annually [1]. While a number of effective medical interventions have been developed, including drugs against various targets, the option of stimulating regenerative pancreatic beta-cell growth therapeutically has long been sought after [2]. Given the more usual pattern of new drug targets emerging via the gradual accumulation of validation data, the first report in September 2011 that such a function could potentially be achieved by small-molecule inhibitors of beta-site APP-cleaving enzyme 2 (BACE2) came as a surprise but welcome new entry into the drug target arena [3]. There were in fact, two indirect precedents. The first, reported in 2005, was that levels of transmembrane protein 27 (TMEM27 or Collectrin) on pancreatic beta-cells were associated with increased islet mass in mice and that was abolished if this protein was proteolytically cleaved [4]. The second was the location of BACE2 in secretory granules of mouse and rat pancreatic beta-cells, reported in 2008 [5]. The 2011 paper identifying BACE2 as the major TMEM7 cell surface secretase connected and extended these observations [3]. This landmark publication also showed that BACE2 knockout mice exhibited islet beta-cell proliferation and cleared blood glucose more efficiently than wild-type controls. Additional in vivo proof-of-concept experiments in this paper showed that a BACE2-specific inhibitor (from an early Roche patent, see below and Figure 1) stimulated the growth of new pancreatic beta-cells in mice.
At this point, it is relevant to review the history of the beta-site APP-cleaving enzyme 1 (BACE1) and BACE2 proteins. They share 50% sequence identity and form a distinct protein subfamily of membrane anchored aspartyl proteases having 25% sequence identity to the cathepsins. In 1999, four groups independently reported the identity of the amyloid precursor protein (APP) beta-secretase as BACE1, thus publically starting the race to develop small-molecule inhibitors of this enzyme for Alzheimer’s disease (AD) [6-9]. Within a year, additional publications reported the cloning of BACE2 [10-13]. In fact, sequence patents for both enzymes arising from the collaboration between Human Genome Sciences and what was then SmithKline Beecham were published before the journal articles [14,15].

The pronounced publication bias in favour of BACE1 is because of its status as an AD drug target. This is reflected in the PubMed gene name retrieval ratio. Queries for BACE1 (but not BACE2); BACE2 (but not BACE1) indicate a ratio of 1883:14 (January 2013). This is further emphasised by intersecting with the term ‘inhibitor(s)’ as a filter for drug research reports. This gives 889:4, but two are false-positives, making the inhibitor publication ratio for the two enzymes as approximately 400:1. The global efforts to discover and progress BACE1 inhibitors over the last decade have thus given rise to nearly 900 inhibition patents from both the academic and commercial sectors. This includes no less than 208 PDB (Protein Data Bank) entries with inhibitor ligands and several candidates expected to enter Phase I clinical trials in the near future [16]. There is also a review of medicinal chemistry patents from 2006 to 2011 [17]. While there are concerns that reducing neuregulin cleavage in synapse development could be problematic for BACE1 inhibition, there are reports of inhibitor design to reduce this possible side effect [18].

### Article highlights.

- Lowering of mouse glucose levels by beta-site APP-cleaving enzyme 2 (BACE2) inhibition was reported in 2011.
- BACE2 is now declared as a new human diabetes mellitus type II (T2DM) target.
- The Roche BACE2 assay patent and first inhibitor patents were published in 2010.
- First Novartis BACE inhibitor patents were published in 2012.
- Combined BACE1 and/or BACE2 patents have appeared from three companies.
- Over 10 chemical series with low nM BACE2 inhibitors have been specified.

This box summarizes key points contained in the article.

2. Methodology for document retrieval and structure identification

This article was researched using open sources, with the exception of subscription access for journal papers. Patents were initially retrieved using the Espacenet and WIPO query interfaces followed by title and number cross-checks on Google [19,20]. The Espacenet INPADOC listings were used to check for the earliest WO publications for family members. The basic keyword queries used for the patent office indexed fields (e.g., BACE2 AND inhibitor AND diabetes) were re-run against the complete text in FreePatentsOnline and the Biological Patent Abstract subset of Europe PubMed Central, but no additional true-positives were detected [21,22]. As always with patent searching, false-negatives (i.e., cryptic BACE2 inhibitor filings for diabetes) cannot be completely ruled out if applicants chose to omit protein names and synonyms from patent document sections typically indexed for searching. However, because the deliberate non-specification of an established target name could be problematic for claim examination, this can be considered unlikely.

Chemical structures for example numbers linked to data in patents were identified via SureChemOpen and inhibition data tables were cross-checked in the original document PDF [23]. All structures were checked against PubChem and by Google InChIKey searches [24]. PubChem Compound Identifiers (CIDs) were successfully identified for all structures included in this report [25]. In the case of salts, the CID for the parent structure has been selected. In PubChem the CID records will link through to all sources, including patent documents in SureChemOpen and/or SCRIPDB, journal articles via ChEMBL, abstracts via PubMed and PDB structures via MMDB. Subscribers to the Thomson Pharma web application can open this via CIDs that include a link. Web addresses of the general form ‘/pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=xxxxxxx’ can be used to display any CID number and the associated links.

3. BACE1 versus BACE2 versus and/or patents

Given what has now become an intertwining of drug discovery for the two targets, the consequent challenges in patent search specificity need to be outlined. A low-specificity search of the EBI biological patent abstracts with just BACE1, BACE2 or both, returns a ratio of 533:40:20, reflecting the predominance of BACE1 reports related to AD. More specific searches across the other sources mentioned, using ‘BACE2 (or -2)’ with ‘diabetes’ keyword searches, retrieved 19 patent families as distinct application titles and unique WO numbers that mention BACE2 in a diabetes context as part of the abstract or description. These were published between June 2010 and December 2012. However, on the basis of data content, only six of these can be classified as likely bona fide BACE2 primary target applications. Others can be classified as ‘and/or’ BACE1/BACE2 patents. Criteria used for the inclusion of such mixed filings in this review were: i) the presence of an extended diabetes section in the description; ii) extensive BACE2 inhibition data with approximate parity to the BACE1 data; iii) inclusion of at least some sub-100 nM BACE2 IC₅₀s and iv) at least a proportion of the compounds appeared to be BACE2-selective. Table 1 includes
Figure 1. Structures used as BACE2 inhibitors in the literature and assay patents.

Table 1. Reviewed patents that include BACE2 inhibition data for diabetes.

| Title and notes                                                                 | Number          | Publication date | Assignees                  |
|---------------------------------------------------------------------------------|-----------------|------------------|----------------------------|
| 5-Substituted iminothiazines, mono- and dioxides as BACE inhibitors,            | WO2012139425    | 18 Dec 2012      | Schering                   |
| compositions, use (BACE1 & 2)                                                   |                 |                  |                            |
| 1,3-Oxazines as BACE1 and/or BACE2 inhibitors                                    | WO2012139993    | 18 Oct 2012      | Roche & Siena              |
| 1,4-Thiazepines/sulfones as BACE1 and/or BACE2 inhibitors                       | WO2012119883    | 13 Sept 2012     | Roche                      |
| BACE2 inhibitors for the treatment of metabolic disorders                        | WO2012095521    | 19 Jul 2012      | Novartis                   |
| Novel heterocyclic derivatives and their use in the treatment of                | WO2012095469    | 19 Jul 2012      | Novartis (Aurigene)        |
| neurological disorders (BACE1 & 2)                                              |                 |                  |                            |
| BACE inhibitors for use in the treatment of diabetes (BACE2)                    | WO2012028563    | 08 Mar 2012      | Roche                      |
| Amino oxazine derivatives (BACE2)                                               | WO2011070029    | 16 Jun 2011      | Roche                      |
| 2-Amino-5,5-difluoro-5,6-dihydro-4H-oxazines as BACE1 and/or BACE2 inhibitors  | WO2011069934    | 16 Jun 2011      | Roche & Siena              |
| 2-Aminodihydro[1,3]thiazines as BACE2 inhibitors for the treatment of diabetes   | WO2011029803    | 17 Mar 2011      | Roche                      |
| 3-Amino-5-phenyl-5,6-dihydro-2H-[1,4]oxazine derivatives (BACE1 & 2)            | WO2011020806    | 24 Feb 2011      | Roche & Siena              |
| Dihydropyrimidinones for use as BACE2 inhibitors                                 | WO2010128058    | 11 Nov 2010      | Roche                      |
| Screening assay for metabolic disease therapeutics (sic) (BACE2)                | WO2010063718    | 10 Jun 2010      | ETH-Zurich                 |
| Screening assays for the identification of BACE2 inhibitors                      | WO2010063640    | 10 Jun 2010      | Roche                      |

Notes appended to titles indicate the target focus.
The company Aurigene was given as the affiliation of inventors included in a Novartis patent.
ETH: Eidgenössische Technische Hochschule Zürich; Novartis: Novartis AG Switzerland; Roche: F. Hoffmann-La Roche AG Switzerland; Schering: Schering Corp. USA; Siena: Siena Biotech S.P.A. Italy.
the 13 patents specifying BACE2 inhibitors selected for chemistry analysis in order of their publication dates. While five additional patents mention BACE2 in a diabetes context as part of the abstract or description (in addition to BACE1), they were found either have no BACE2 inhibition data, or low-potency structures likely to be cross-screening results from BACE1-directed patents.

3.1 BACE2 inhibitors in the literature and assay patents

Before describing the medicinal chemistry patents, the precedents in the literature and earliest assay filings can be reviewed. Before the Stoffel team’s 2011 paper, there were no reports of specific BACE2-directed inhibitors in the absence of any therapeutic rational. There were, however, publications that included cross-screening data of BACE1 inhibitors against BACE2. In the ChEMBL database this is formally recorded as a BACE1:BACE2 compound data ratio of 3014:529 extracted from a corpus of ~ 50,000 medicinal chemistry publications [26]. A few of the latter, such as CHEMBL590829 (CID 16051698), were not only potent against BACE2 but also the ‘right way round’ (i.e., with IC50 of 10 nM for BACE2 and 20 nM for BACE1). Another precedent is a 2006 paper from Novartis describing the crystal structure of human BACE2 in complex with a hydroxyethylamine transition-state inhibitor (PDB code 2ewy) but without inhibition data [27]. The ligand, CID 11963503, has no patent matches, even though it is implicitly included in their filing on the crystal structure [28]. Notably, the patent examiner designated this filing as non-inventive based on obviousness from BACE1 structures.

The earliest declarations of BACE2 inhibition data in the context of diabetes drug discovery are the two assay patents that were published on 10 June 2010 [29,30]. However, at this early stage prior-art BACE1 inhibitors were used for assay calibration. The filing by the academic group (ETH-Zurich) used a reference compound (CID 5287532) published by Merck in a BACE1 PDB structure in 2006 [31]. The Roche assay patent also used a BACE1 PDB ligand but the structure (CID 16040323) had its origins in an AstraZeneca/Astex...
The IC$_{50}$ was reported as 84 nM for BACE2 in the Roche assay patent [29]. This filing is significant not only because it circumscribes the structure–activity relationships (SAR) for the ensuing Roche screening results but may also constrain freedom to operate for other commercial entities. They describe fluorescence resonance energy transfer (FRET) assays using cleavage of peptide substrates based on both TMEM27 and (because of homology in the transmembrane anchoring region) angiotensin cleaving enzyme 2 (ACE2). They also describe an ELISA assay for soluble TMEM27 released, by BACE2 cleavage, into the supernatant of cells transfected with both proteins. They have also developed a pancreatic beta-cell proliferation assay as a third strategy for inhibitor assessment. Both the FRET peptide and TMEM27 release assays are used in the Roche medicinal chemistry patents, but results from the beta-cell proliferation assay have not featured in these so far.

The ETH assay patent describes a more extended set of biochemical characterisations but one of the claims covers measurement of shed (i.e., BACE2-clipped) and full-length TMEM27 from a cell line incubated in the presence of inhibitors [30]. The document includes substantially the same data that appeared in the eventual journal publication (see below) but Roche appear to have used only their own assays in patenting. While Roche are not explicitly connected to the patent from the academic team, their patent attorneys are the filing agents and were thus presumably responsible for synchronising the publication dates.

The first mouse plasma glucose-lowering experiments were reported in the Stoffel team’s 2011 paper [3]. The proof-of-concept ‘compound J’ had reported BACE1 and BACE2 inhibition IC$_{50}$s of 180 and 6 nM, respectively. However, the structure is specified only as a low-quality image in the supplementary data. There was also some ambiguity over its origin, because the authors referred to a Shinogi patent that included only a chirally related structure (example 1043) reported as a 27 nM BACE1 inhibitor [33]. Searching indicates the structure was in fact example 1 (CID 50938551) reported as having a 9 nM IC$_{50}$ against BACE2 in one of the early Roche patents reviewed below [34]. The five structures used as early BACE2 inhibitors are shown in Figure 1.

### 4. Patent evaluations

#### 4.1 First Roche applications

Within 6 months of the assay patents, the first Roche BACE2-only application appeared in November 2010, based...
on a dihydropyrimidinone scaffold developed by two inventors [35]. Beyond the usual descriptions of synthesis and analytical chemistry data, the core of this application is a table (pages 108–9) of 15-point BACE2 IC$_{50}$ data for the 130 examples, as both IUPAC names and images (but specified as salts). All of these are claimed. In the IC$_{50}$ table for the FRET peptide assay, 30 examples are recorded at 20 nM or below. The most potent IC$_{50}$s include examples 20 (CID 46944265) 6 nM, 44 (CID 59570593) 7 nM, 47 (CID 49818770) 8 nM and 122 (CID 50992405) 10 nM (Figure 2).

There is only one sentence referring to BACE1 and AD in the document and no in vivo data were included. The February 2011 Roche/Siena oxazine patent includes a new assay for Kd values for the binding of compounds to immobilised enzymes using surface plasmon resonance (SPR) [36]. This means there is a table of Kd and IC$_{50}$ values for BACE1 and BACE2 with 99 examples on pages 59–62 (with some data gaps). The most potent for BACE2 (and lower than the BACE1 values) by IC$_{50}$s are example 98 (CID 50991498) 1 nM, 40 (CID 50992222) 2 nM, 99 (CID 50991499) 3 nM, 51 (CID 50992405) 5 nM (Figure 3).

The next Roche series, the aminodihydro thiazines, was published in March 2011 [34]. This is different to the previous dihydropyrimidinone application in several ways. As a smaller document, there are only 19 examples but 6 inventors. However, only the first two have any potency in the peptide substrate assay, with BACE2 IC$_{50}$ values of 8 and 9 nM, respectively, for example, 1 (CID 50938551) and 2 (CID 69922561). The next values in the series, 6 (CID 6992184) and 18 (CID 51001972) are already down to 440 and 474 nM, respectively, with most of the remaining being between 1 and 50 µM (Figure 4). It turns out, not only that example 1 is ‘compound J’ from the 2011 paper (Figure 1) but it was also used for measuring TMEM27 cleavage reduction in isolated human pancreatic islets, as well as glucose metabolism effects in Zucker Diabetic Fatty rats in vivo. The results for these
experiments are given in a set of figures from page 52 onwards. Some of these include dosage steps for compound J, with liraglutide (CID 44147092) as a positive control, but no analogues for SAR. The biological data in this patent thus complement the mouse data from the 2011 paper for compound J.

Within 3 months of the aminodihydro thiazines, two additional Roche patents were published simultaneously in June 2011. The BACE2-only amino oxazine series had a single inventor [37]. The core of this patent is also an IC50 table (pages 72–3) but, rather than a peptide substrate assay, data are reported for the inhibition of human TMEM27 cleavage by endogenous BACE2 in a rat cell line via an ELISA. This is arguably a more disease-relevant assay from the Roche assay patent but is actually using rat enzyme. The 65 structures tested vary from 1 nM to 9 µM IC50s. The most potent are examples 24 (CID 67235275), 26 (CID 53241550), 29 (CID 53241553) and 1 (CID 53241150). For the second oxazine series from June 2011, Siena Bio-tech were co-assignees and this was the first Roche ‘and/or’ patent for both targets [38]. Using the same rat BACE2/human TMEM27 assay, the IC50 table on page 42 includes 83 examples with BACE1 data but only 62 BACE2 measurements. Of these examples, eight were selected for pharmacological screening in a number of assays, including hERG (human ether-à-go-go-related gene), P450, in addition to cathepsin D and E cross-reactivity (page 60). The following structures, common to both data sets, are listed in order of their BACE2 IC50s. Examples include: 1 (CID 53241709) 35 nM, 5 (CID 53241713) 15 nM, 8 (CID 53241825) 71 nM and 11 (CID 67234976) nM (Figure 6).

Moving on in sequence, the Roche ‘BACE inhibitors for use in the treatment of diabetes’ appeared in March 2012 [39]. This describes a series of N-[1-benzyl-2-hydroxy-3-arylamino-propyl]-isophthalamides and N-[1-benzyl-2-hydroxy-3-heteroarylamino-propyl]-isophthalamides (sic). The results are again generated using the cell-based human TMEM27 rat BACE2 assay and a table of 75 BACE2-only IC50s is presented (pages 29–30). While nominally the most potent compound, the 0.1 nM reported for example 41 (CID 56846820) is probably outside the limits of the assay and thus may be a typo for 1 nM. Other potent analogues include example 63 (CID 56846996) IC50 1 nM, 43 (CID 57512748) 3 nM and 51 (CID 56846933) 4 nM (Figure 7). Substituted isophthalamides have already been

Figure 5. Roche amino oxazines from WO2011070029.
4.2 Novartis

The first competition to Roche appeared as a double publication in July 2012. We can look at the BACE2-only patent first [40]. This big filing of 596 pages has a single inventor for an oxazine series. There are 274 examples in the IC50 table (pages 432 – 6) with results generated from an unspecified fluorescent APP-derived peptide assay. The use of a BACE1 substrate peptide for a BACE2 assay means absolute values cannot be directly compared with the Roche results. However, regardless of which assay types they may have implemented internally for this project, their choice of what to actually publish may have been contingent on freedom-to-operate constraints as a consequence of the Roche assay patent. A selection of the most potent results includes example 66 (CID 50914981) 6 nM, 92 (CID 50913216) 4 nM, 85 (CID 50913073) 8 nM and 207 (CID 53262203) 3 nM (Figure 8). Examination of the document also revealed a crystallisation experiment for example 152 (CID 58118888) that had no activity data (page 261). The first assumption was that this could have been an in vivo lead but the structure turns out to be their own prior-art from a Novartis BACE1 oxazine patent published in January 2011 [41]. This includes the same crystallisation data with the explicit mention that this could be a preclinical candidate (although, as in many other cases, ‘BACE’ is used as a synonym for BACE1 in this document). The observation that the Markush scaffold substitution points are the same is further indication of a cross-over. The simplest explanation is there has been some ‘re-cycling’ of compounds from the 9943 BACE1 patent into the later 5521 BACE2 patent. This might also explain the anomaly of the prodigious single-inventor output for the 274-example BACE2 patent but the apparent need for 15 inventors on the earlier 185-example BACE1 effort. The priority date of July 2009 also implies that 9943 was a bona fide BACE1-only filing.

Possibly emulating the Roche precedent, the second of the Novartis July 2012 patents, that includes Aurigene inventors, is also an ‘and/or’ filing extending the oxazines for both targets [42]. The IC50 tables (pages 36 – 9) present, in sequence BACE1, BACE2 and Ab 1-40 release data for 48 examples. The most potent BACE2 data include example 46 (CID 57524448) 5 nM, 38 (CID 70355171) 5 nM, 24 (CID 70355131) 10 nM and 11 (CID 70356221) 12 nM (Figure 9).

4.3 Latest Roche output

Two months after Novartis, Roche also published another ‘and/or’ patent in September 2012 on thiazepine sulfones. While this includes 14 examples, only 10 of these have both BACE1 and BACE2 IC50 results (pages 35 – 8) [43]. As a combined table this usefully juxtaposes specificity data but it should be noted that the two cellular assays are different (i.e., A-beta peptide release vs the TMEM27 clip), so potency ratios are not directly comparable. Nevertheless, examples can be picked out in three categories. The first are approximately equipotent, such as example 3 (CID 60171592) with IC50s against BACE2 of 5 nM and BACE1 of 3 nM, together with example 7 (CID 60165763) at 2 nM and 4 nM, respectively. A second set showed BACE2-selectivity, such as example 6 (CID 60171716) at 9 nM for BACE2 and 140 nM for BACE1. The third category, BACE1-selectivity, was represented only by example 1 (CID 60171590) at 167 nM for BACE2 and 6 nM for BACE1 (Figure 10).
The latest patent from Roche/Siena, another oxazine series, was published in October 2012 [44]. On pages 38 – 42, BACE1 and BACE2 assay results are included for some but not all 52 compounds. In nearly all cases, the specificity is towards BACE1 but there are also some potent results for BACE2. In order these are: example 1 (CID 66558600) 1 nM, 3 (CID 66557906) 7 nM, 8 (CID 66558605) 6 nM and 7 (CID 66558604) 10 nM (Figure 11).

4.4 Schering
As the last patent in this review, the application by Schering represents the third pharmaceutical company to extensively

Figure 7. Roche isophthalamides from WO2012028563.
specify combined BACE1 and BACE2 inhibition, in this case using an iminothiazine series published in December 2012 [45]. This document has been made deliberately difficult for SAR navigation by complex table nesting and the mix of ranged, plus discrete, $K_i$ values for BACE2 specified only in text (pages 187 – 90) outside the structure tables that include the BACE1 data. As in the Novartis case, a BACE1 substrate is used for the BACE2 assay. A number of moderately potent BACE2 inhibitors are exemplified including 9ap (CID 68111365) 4 nM, 9bz (CID 66563157) at 13 nM, 11 (CID 66563594) 13 nM and 9cf (CID 68111462) at 40 nM (Figure 12). As for the Novartis case above, there are indications that these data may have been added on to what started out as BACE1 project. In particular, the SureChemOpen searches indicate structures-in-common with an earlier US-only (i.e., no INPADOC family) BACE1 patent on the same chemical series [46]. This also mentions BACE2 as a diabetes target and includes some low potency data (e.g., example 34, CID 68111340, 220 nM $K_i$) but this may just be cross-screening. In addition, the 2009 priority date precedes the 2010 BACE2 assay patent cited in the published application.

5. Expert opinion

The patents above have certainly initiated the race to develop aspartyl protease inhibitors for diabetes. BACE1 and BACE2 thus now have the unusual attributes of being a pair of paralogous human drug targets with separate major therapeutic indications, in different tissues, with the same enzyme mechanism, but whose initial validation is separated by over a decade. Considering the major unmet medical need in both AD and T2DMs, the progression of inhibitors towards becoming
first-in-class medicines in either case, is to be earnestly hoped for. However, by any criteria BACE2 is a ‘young’ target so additional validation data will be important, particularly where it can provide independent experimental support for the therapeutic hypothesis. The Stofel group has already extended their research with a recent study on the intramolecular features of TMEM27 that regulates its processing by BACE2 [47]. Notwithstanding, other studies have suggested additional direct or indirect mechanistic relationships of both enzyme activities to glucose metabolism. The earliest of these reported that BACE2 plays a role in the insulin receptor trafficking in pancreatic beta-cells [48]. A more recent publication, suggesting that BACE1 may also be involved in glucose homeostasis, is supported by data from BACE1 knockout mice, together with experiments showing BACE1 inhibition in a skeletal muscle cell line increased glucose uptake and enhanced insulin sensitivity [49].

The availability of genetically modified animal models and general tractability of in vivo diabetes studies should make resolving these biochemical complexities and consolidating BACE2 target validation somewhat easier than has been the case for BACE1 and AD. Another emerging advantage for BACE2 is a choice of tool compounds that can already be picked out from the patent literature, including structures from this review. As for BACE1, the BACE2 mouse knockout seems to be devoid of major functional consequences [50]. While this is reassuring for an inhibitor program, the subtle indications of neuronal dysfunction that emerged some years later for BACE1 knockouts emphasises the need for detailed mouse phenotyping on the BACE2 equivalent. In addition, recently reported initial characterisation of zebrafish BACE1 and BACE2 knockouts will need to be explored further to see how closely they phenocopy their mouse counterparts [51].

Given the scale of human Genome Wide Association (GWAS) studies undertaken in diabetes it would be a bonus for target validation if rare variants (since common ones should have been detected by now) related to gain or loss of function for BACE2 and/or TMEM27 might be detected in new studies or meta-analyses. In fact, despite extensive GWAS surveys for AD, no such genotype effects have ever been detected for BACE1. This is in contrast to the many presenilin variants (PSEN1 and PSEN2) with early-onset AD associations that have helped validate gamma-secretase as a target [52]. However, encouraging new indirect genetic support for BACE1 was reported in August 2012. A mutation in APP, A673T, was found to be protective against AD and age-related cognitive decline, acting via reduced beta-secretase cleavage [53].

The fact that Roche already had BACE1 expertise (including medicinal chemistry patents with priorities back to 2003 and an assay filing back to 2001) in conjunction with their fortuitous academic collaboration, has certainly given them a head start for BACE2 inhibition. Given their own assay patent has a priority date back to December 2008, it can be assumed that they are at least 4 years into the project. Nevertheless, the filings described above, particularly from the paucity of in vivo pharmacological data, do not suggest they have advanced to clinical candidate selection, even within the scope of the Markush enumerations. This is, of course, difficult to tell, but there are no published follow-on filings for scale-up synthesis or formulations that are the typical hallmarks of clinical candidates.

The new scenario of parallel tracking for these two targets will have interesting consequences. One of these is the precedent, set by Roche, for the ‘and/or’ combined BACE1/BACE2 patents where Novartis and Schering have now followed suit. This seems likely to continue and will present patent informatics challenges (as for any filings that include data

Figure 9. Novartis oxazines from WO2012095469.
for multiple plausible primary targets). On the basis of document content per se, it is difficult to discern the real ‘intent’ of the double-target filings that may simply represent opportunistic reciprocal cross-screening (not that this is necessarily problematic in intellectual property terms). This is particularly the case for those ‘and/or’ patents that appear to be evenly weighted in the sense that they include full descriptions for both diseases, used two assays (in the Roche cases) and present extended result sets for both targets. The only external filter that could be applied, is where brain-penetrant properties for a chemical series could imply these were being designed primarily against BACE1. However, inspection also reveals clues regarding BACE1-centricity. One of these is that the 2007 collaboration announced between Siena Biotech and Roche was specifically for neurodegenerative diseases. Therefore, it is a plausible assumption that some of the Roche/Siena ‘and/or’ patents may have started out as BACE1 projects engaged in by the large collaboration team. It remains to be seen as to whether the chemical series used in these ‘and/or’ patents eventually fork into separate lead optimisation campaigns and new patent filings more obviously specific for each of the targets.

Given the more usual precedent of pharmaceutical companies finding their targets from the literature, this head start from a single company is unusual. Notwithstanding, the competing Novartis and Schering patents cite the Roche 2010 assay patent as a precedent. Thus, anyone else doing a competitive intelligence trawl may also now have BACE2 on their target selection radar screen. In principle, any medicinal chemistry team with BACE1 experience, combined with institutional access to experimental diabetes models, could fast-follow (except they may need to invent their ‘own’ BACE2-specific assays to specify in their patents). This could be accomplished by adding BACE2 to their target portfolios, or even, if they perceived a greater chance of success, switching to it and closing their BACE1 program (given the scaling-back of neuroscience R&D by pharmaceutical companies this may have already happened).

There are also reasons to believe that accumulated collective BACE1 experience could have an accelerating effect on BACE2 lead development (i.e., telescoping the 12 years already needed for the first clinical candidates for AD). One of these is obviously the re-use of scaffolds, libraries, fragment-based approaches and other lead design strategies (although this is likely to result in a patent thicket where finding novel series for either enzyme will become increasingly difficult). Another advantage BACE2 lead development will have is not needing to cross the blood–brain barrier. Decisions regarding acceptable cross-reactivity between the paralogous proteases may thus need to be revised as a result of the newly-discovered BACE2 functions. Hitherto, the choice of selectivity in vitro has probably been set at some arbitrary threshold (e.g., IC50 ratios < 100), or even not tested at all, simply because the consequences of BACE2 inhibition were unknown. This may also explain the relatively low level of published cross-screening results that (up to 2010) were recorded as a BACE1:BACE2 ratio of 5459:414 from patents and papers [54]. Whereas, as mentioned above, the equivalent ratio extracted from papers in ChEMBL (up to 2012) is now 3014:529. These considerations also present new options for any organisation planning clinical studies for a BACE1 inhibitor, particularly if the compound also proved to be an effective BACE2 inhibitor in vitro. It can be predicted that: i) BACE2 specificity will now be retrospectively tested if this was not already done, ii) any well-tolerated BACE1 leads with significant BACE2 cross-reactivity could be high-dose tested for glucose-lowering effects in rodent models and iii) for BACE1 inhibitors entering Phase I clinical trials, patient blood samples could be permissioned for glucose and TMEM27 measurements.
Figure 11. Roche/Sienna oxazines from WO2012139993.

Figure 12. Schering iminothiazines from WO2012139425.
Further predictions can be made for future therapeutic patenting strategies for BACE2, some of which might also influence new BACE1 filings. The first would be an increase in BACE2-directed applications across the board as more organisations re-design for the paralogous specificity switch (i.e., target-hop). The second would be that cross-screening is now more likely to be reciprocal and complete (i.e., all BACE1 compounds screened against BACE2 and vice versa). The third, in the light of the new role for BACE2, is a re-assessment of specificity requirements. For example, a potential side effect from BACE1 inhibitors of moderately decreasing glucose levels could even be seen as an asset rather than a liability. Consequently, the optimisation of ADMET parameters need not be constrained by a need to minimise BACE2 cross-reactivity. This principle also applies vice versa in that concerns regarding cross-reactivity of BACE2 inhibitors against BACE1 could be ameliorated because these compounds will not be selected for brain-penetrant properties (i.e., they should not be able to interact with BACE1 in the first place). In connection with this, a recent report has suggested another possible role for BACE2 in the degradation of amyloid beta-protein [55]. While the relevance of this for therapeutically modulating human neuronal amyloid deposition in AD remains to be established, if confirmed, this becomes another argument for ensuring BACE2 inhibitors are not brain-penetrant. A fourth prediction (as a logical corollary of the third) is the possibility of polypharmacology whereby dual inhibitors might be designed. This is obviously speculative but, given the established (and increasing) comorbidity between AD and diabetes this option, or even drug combinations against both targets, may not stay completely beyond consideration [56].

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Declaration of interest

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Note added in proof

An updated version of Table 1, including some new 2013 BACE2 patents, can be accessed at http://figshare.com/articles/BACE2_patents/643815.

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