P082

# GAMMA-SECRETASE MODULATORS SHOW SELECTIVITY FOR GAMMA-SECRETASE MEDIATED Aβ PRODUCTION

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## Background and objectives

The process of Aβ amyloidosis plays a pivotal role in the onset of Alzheimer's disease (AD) and starts decades prior to symptomatic disease. It is thus conceivable that an Aβ-targeting therapy would be most beneficial as a preventive therapy initiated during preclinical AD. The major constituent of Aβ amyloid is the post-proteolytical Aβ peptide, which stems from γ-secretase mediated processing of amyloid precursor protein (APP). γ-Secretase has a large number of substrates and is involved in many contexts of cell signaling. γ-Secretase modulators (GSMs) represent a promising class of orally available Aβ modulatory agents for the treatment of early AD. In this study we have asked what impact GSMs may have on other γ-secretase reactions, with emphasis on EphA4, EphB2 and E-cadherin signaling.

## Methods

Expression constructs encoding N-terminally truncated and FLAG-epitope tagged EphA4, EphB2 and E-cadherin were expressed in murine cells lacking presenilin (PS) expression (BD8 cells) and in human HEK293 cells. Cells expressing APPswe and C99-GVP (direct substrate of Aβ) had been generated before. γ-Secretase mediated intracellular domain (ICD) formation and secretion of Aβ and Aβ-like peptides were analyzed in cell-based assays using a reporter gene system and by a combined immunoprecipitation/mass spectrometric analysis, respectively. Ectopic expression of PS1 in BD8 cells and the γ-secretase inhibitor L685,458 were used in experiments to determine the γ-secretase dependency of different reactions. Three structurally distinct GSMs were used to explore the impact of GSMs on the intramembrane processing of EphA4, EphB2 and E-cadherin.

## Results

Cells overexpressing EphA4 and EphB2 resulted in a PS and γ-secretase dependent generation of ICDs and Aβ-like peptides (Fig 1). In contrast, E-cadherin overexpression resulted in γ-secretase independent formation of ICD and Aβ-like peptides (Fig 1A and data not shown). The amount of ICD generated from EphA4, EphB2, E-cadherin and C99 expressing cells were unaffected by all GSMs tested (Fig 2A). None of the secreted Aβ-like peptides generated from EphB2 or E-cadherin expressing cells were modulated by the GSMs (Fig 2 and data not shown). Only the GSM AZ4126 modulated EphA4 derived Aβ -like peptide formation (Fig 2D), but with an order of magnitude lower potency as compared to its Aβ modulatory effect (Fig 3).

## Conclusion

Our studies confirm that γ-secretase mediates intramembrane processing of EphA4 and EphB2 and show that it results in multiple secreted Aβ-like peptides. Our data also suggest that E-cadherin could be subject to intramembrane processing by an enzymatic activity that is distinct from γ-secretase. Importantly, our data demonstrate that GSMs, developed for targeting APP processing and Aβ production, are selective for Aβ modulation. The molecular basis for this selectivity remains unknown, but strongly support the development of a GSM therapy for the targeted lowering of amyloidogenic Aβ production in the preclinical, pre-symptomatic phase of AD.

**Figure 1.** γ–Secretase dependent EphA4 and EphB2 ICD (A) and Aβ-like peptide (B and C) generation. PS deficient cells in A. and HEK293 cells in B and C. PS1 (=PS1 overexpression), L685,458 (= γ-secretase inhibitor treatment)

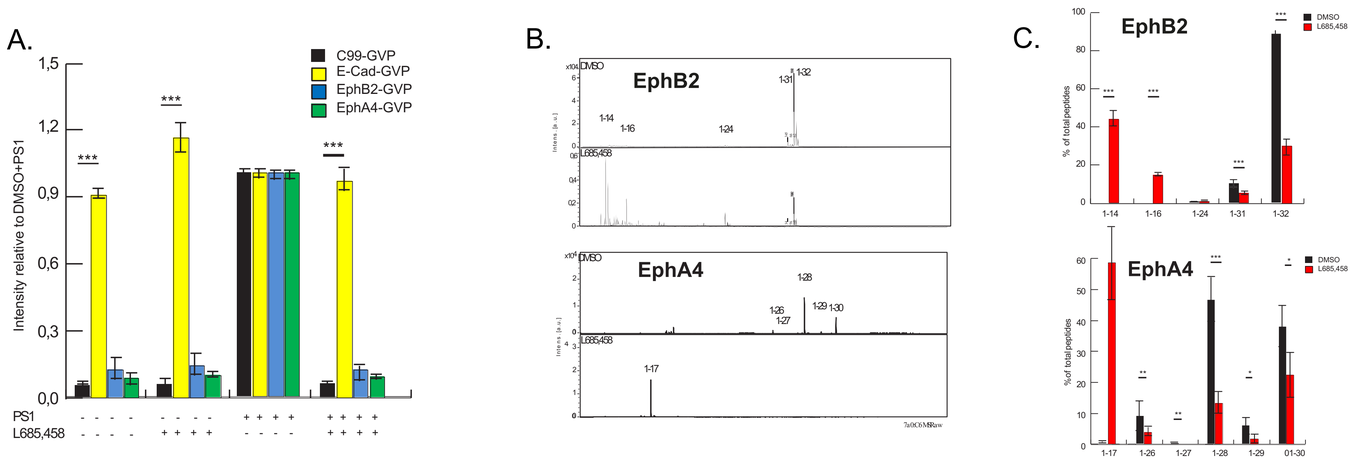



**Figure 2. T**he impact of GSMs on the γ-secretase mediated ICD (A), and Aβ-like peptide formation in cells overexpressing N-terminally truncated and epitope tagged EphB2 (B) and EphA4 (C).

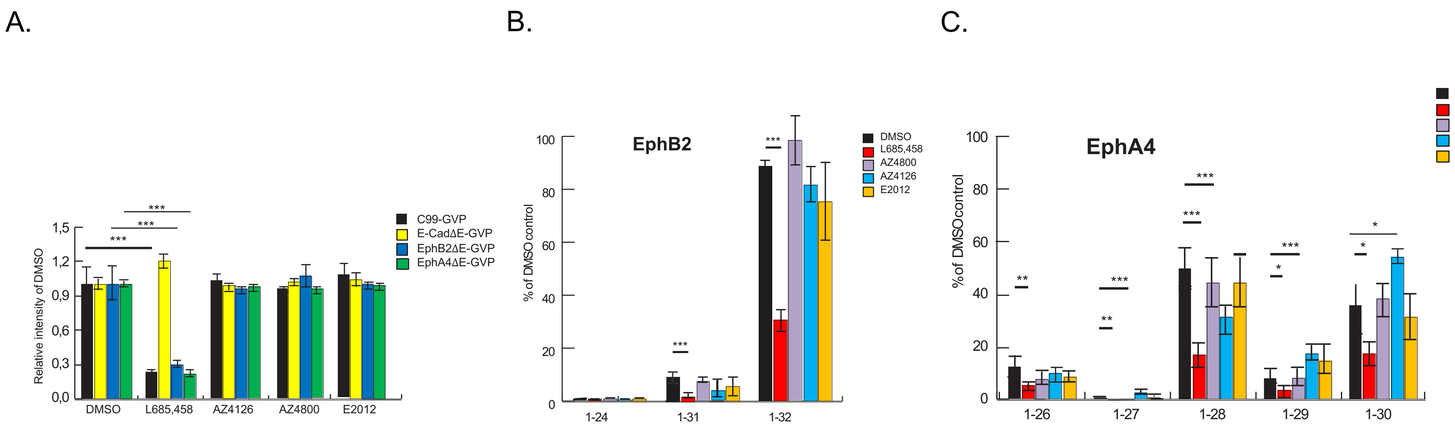
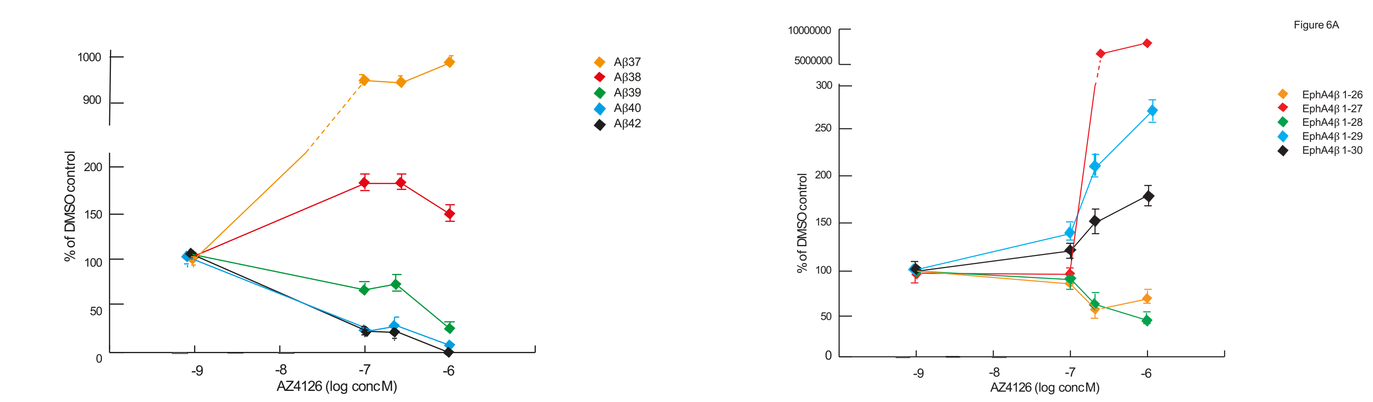



**Figure 3.** The GSM 4126 shows a more potent modulation of Aβ (A) as compared to EphA4 Aβ-like peptide formation (B)




**Key findings:**

1. γ-Secretase processing of EphB2 and EphA4 results in the release of multiple Aβ-like peptides
2. GSMs developed for Aβ modulation appear not as effective on modulating γ-secretase mediated EphB2 and EphA4 processing
3. The GSM AZ4126 modulates the pattern of EphA4-derived Aβ-like peptides differently as compared to its effect on Aβ

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