CNS drug development remains challenging when comparing development times (clinical phase and approval phase) in various therapeutic areas. Kallin demonstrated that drug development in the field of CNS is the longest, taking approximately 10 years compared to any other disease and with an average of 7.6 years in Oncology. In addition, the approval success rate remains only 8.2% in CNS where in Oncology this rate is close to 15% (DiMasi, 2010). In 2009, Lon Schneider tried to explain these failures by analyzing 23 clinical trials of more than 18 months. He stated that “at least two things need to occur to improve chances for detecting efficacy with current outcomes. A greater proportion of the placebo samples need to meaningfully worsen on the primary outcome scale, and the drug group needs to improve over baseline to overcome the broad variances in change” (Schneider, 2009). In 2008, R. Becker stated that “unfortunately, in spite of their using of larger numbers, higher levels of variance appear to account for the failure of two trials to reach statistical significance” (Becker, 2008 Auguart).

Therefore it is critical to expand the identification and use of clinically relevant biomarkers which can contribute to the renewal of AD drug development. The objective is to show how, a non invasive and reproducible diagnostic test, AclarusDx™, could increase the success rate of clinical trials by reducing the variability in patient inclusion. Furthermore, the use of the human Genome-Wide SpliceArray™ (hGWAS) technology used for the development of AclarusDx™ test, can bring to pharmaceutical companies the capacity to identify at completion of clinical phase II studies, an additional signature to distinguish potential responders from non responders to the drug being developed. In the EHT0202 study, we identified specific expression profiles for decliners and improvers that allowed a clear discrimination of these subgroups at study exit after administration of EHT 0202. This discrimination was specific of EHT 0202 and not detected for the coadministered cholinesterase inhibitors. When the analysis was conducted at the gene level and not at the splice event level, significantly less discrimination between responders and decliners could be made.

Importantly, transcriptomic profiling of the patients prior to treatment allowed to identify blood expression biomarkers which could be potentially useful to prospectively predict patient response.

**Design and content of hGWASA**

<table>
<thead>
<tr>
<th>Exon probes</th>
<th>Intron probes</th>
<th>Matching probes</th>
<th>Mis-match probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long form</td>
<td>Long form</td>
<td>Complete</td>
<td>15%</td>
</tr>
<tr>
<td>Short form</td>
<td>Short form</td>
<td>Partial match</td>
<td>30%</td>
</tr>
</tbody>
</table>

Probe configuration for the human Genome-Wide SpliceArray™ (hGWAS). Potential splice events were identified after expression screening from two public databases. The “exonized events” were selected and probes were designed to detect both the short, exclusive form and the long, inclusive form. F and T probe sets are common in both forms while B, C, and D probe sets are specific to the long form and the E probe set is specific only to the short form. There are two types of discovery probes which include predicted exon-skips and shuiprophs (Zhou et al 2009).

Using the TSP method, 85 probe set pairs were ranked from the group of 170 proteins. Each pair was weight and the probed set pairs were summed to provide a subject-specific prediction score. A grey zone (Coste) was defined to exclude false positive and false negative results in a limited region of the prediction score scale. Grey zone boundaries were determined using a target negative predictive value (NPV) of 0.900 and a positive predictive value (PPV) of 0.750. False NPV and PPV, with the grey zone area defined within the TSP prediction score values of 4 and 1, respectively.

A way to increase the success rate of clinical trials in the field of Alzheimer would be to combine a “Quality Controlled” recruitment phase with AclarusDx™ together with the power of the hGWAS technology to define a signature in order to prospectively identify responders.

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**Conclusions**

**AclarusDx™ test**

The molecular classifier was generated from one cohort of 177 individuals (90 AD patients and 87 controls CT) with no apparent cognitive impairment. 100 patients/subj (50 AD et 50 controls) were issued from the EHT0202 study. Additional samples (40 AD at 37 CT) were issued from a CRO (Precision Med, San Diego, CA).

**Profiling at Gene level vs Probe Set level**

**EHT0202 Phase IIa : Response signatures**

Pilot, randomized, double-blind, 3-arm, placebo-controlled study over a 3-month treatment period with EHT 0202 (40 mg bid, 80 mg bid), or placebo as an add-on therapy to AChEI on 159 mild to moderate AD patients randomized (MMSE : 12-24).

**Primary objective :** Clinical safety/efficacy of EHT 0202 versus placebo.

**Secondary objectives :** exploratory efficacy of EHT 0202 (40 & 80mg bid) on cognitive function (ADAS-cog, NAB, MMSE and additional tests) Analyses of population PK, PK/PD analysis, treatment response versus Apolipoprotein E genotype, sAPPα assay in serum.

Transcriptomic profile (hGWAS) using blood samples taken before study treatment initiation (visit 2) and at study completion (visit 5) are described as below:

- 60 AD patients (20/group) having either improved (10) or declined (10) during the study period with regards to ADAS-cog total score (decrease / increase of at least 3 points on ADAS-cog scale).

**A - Identifying Responders vs. Decliners at study completion**

Patients that improve worse on EHT 0202 have a different gene expression profile to those that decline at study completion.

**B - Baseline expression profiles to discriminate Responders vs. Decliners – prior to treatment**

It is possible to identify baseline expression profiles to discriminate patients who will benefit from EHT 0202 therapy from those that will not.

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**More information is available on : www.exonhit.com**

**CTAD - San Diego, November 3-5th 2011**

Matthew P. Pando, Jennifer Carrière, Laurent Désiré, Pascale Beurdeley, Isabelle Barber

Exonhit SA, Paris, France