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European Prevention of Alzheimer's Dementia Consortium

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D1.6 Interim report on evaluation of biomarker and clinical assessments and outcomes from existing and acquired data

WP1- Scientific challenges

V2.0

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DEFINITIONS

- Partners of the EPAD Consortium are referred to herein according to the following codes:
  - **Janssen**. Janssen Pharmaceutica NV (Belgium)
  - **UEDIN**. The University of Edinburgh (United Kingdom)
  - **UOXF.** Masters and Scholars of the University of Oxford (United Kingdom)
  - **BBRC.** BarcelonaBeta Brain Research Center (Spain)
  - **SYNAPSE.** Synapse Research Management Partners S.L (Spain)
  - **KI.** Karolinska Institutet (Sweden)
  - **VUMC.** Stichting VUmc (Netherlands)
  - **UCAM.** Masters and Scholars of the University of Cambridge (United Kingdom)
  - **MRC.** Medical Research Council (United Kingdom)
  - **BERRY.** Berry Consultants LLP (United Kingdom)
  - **UNIGE.** Université de Genève (Switzerland)
  - **RUMC.** Stichting Katholieke Universiteit (Netherlands)
  - **CU.** Cardiff University (United Kingdom)
  - **CHUT.** Centre Hospitalier Universitaire de Toulouse (France)
  - **IQVIA.** IQVIA, Ltd (United Kingdom)
  - **AE.** Alzheimer Europe (Luxemburg)
  - **EMC.** Erasmus Universitair Medisch Centrum Rotterdam (Netherlands)
  - **APHP.** Hôpital de la Salpêtrière (France)
  - **INSERM.** Institut National de la Santé et de la Recherche Médicale (France)
  - **ULEIC.** University of Leicester (United Kingdom)
  - **IXICO.** IXICO Technologies Ltd (United Kingdom)
  - **ARACLON.** Araclon Biotech S.L (Spain)
  - **FRAUNHOFER.** Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V. (Germany)
  - **Eisai.** Eisai Inc (United States)
  - **SARD.** Sanofi-Aventis Recherche & Développement (France)
  - **NOV.** Novartis Pharma AG (Switzerland)
  - **BI.** Boehringer Ingelheim International GmbH (Germany)
  - **Eli Lilly.** Eli Lilly and Company Ltd (United Kingdom)
  - **HLU.** H. Lundbeck A/S (Denmark)
  - **Takeda EU.** Takeda Development Centre Europe Ltd (United Kingdom)
  - **AC Immune.** AC Immune SA (Switzerland)
  - **Biogen**. Biogen Idec Limited (United Kingdom)
  - **Amgen.** Amgen NV (Belgium)
  - **Pfizer.** Pfizer Limited (United Kingdom)
  - **UCB.** UCB Biopharma SPRL (Belgium)
  - **ARIDHIA**. Aridhia Informatics Ltd (United Kingdom)
  - **ROCHE**. F. Hoffmann - La Roche (Switzerland)
  - **UKK**. University Hospital of Cologne (Germany)
  - **MSD**. Merck Sharp & Dohme (United States)

- **Grant Agreement.** The agreement signed between the beneficiaries and the IMI JU for the undertaking of the EPAD project (115736).
- **Project.** The sum of all activities carried out in the framework of the Grant Agreement.
- **Work plan.** Schedule of tasks, deliverables, efforts, dates and responsibilities corresponding to the work to be carried out, as specified in Annex I to the Grant Agreement.
- **Consortium.** The EPAD Consortium, comprising the above-mentioned legal entities.
- **Project Agreement.** Agreement concluded amongst EPAD participants for the implementation of the Grant Agreement. Such an agreement shall not affect the parties' obligations to the Community and/or to one another arising from the Grant Agreement.

EXECUTIVE SUMMARY

The objective of this document is to present deliverable D1.6. This report will describe the activities undertaken towards evaluation of biomarkers and clinical endpoints using existing data. In order to do that, we will use the v500.0 locked database from EPAD, which includes cross-sectional data on the first 500 EPAD Longitudinal Cohort Study (LCS) participants and longitudinal cognitive data in a small subset. We will describe the performance of the proposed biomarkers, clinical assessments, and outcome measures that were recommended by the Scientific Advisory Groups (SAGs) for use in the LCS. This is comprised of results from the EPAD Cognitive Evaluation (ECE) battery, CSF measures of AD pathology (Aβ, Tau and pTau) recommended by the Fluid Biomarkers SAG and some structural MRI measures as recommended by the imaging SAG.

1. INTRODUCTION

In 2015, D1.3 presented the biomarker recommendations made by the four SAGs. These included the EPAD Cognitive Evaluation (ECE) battery to be performed annually, CSF measures of AD pathology (Aβ, Tau and pTau) to be performed at the baseline visit and at the 1st or 2nd annual visit, MRI, structural and functional, to be performed annually (table1). For a more detailed discussion see D1.3

Currently the EPAD LCS has included over 1000 research participants and in July 2018 we performed the first database lock on baseline data of the first 500 research participants to enter the LCS, where all baseline cognitive and biomarker measures are available. All results presented in this report follow the same scheme: as the total sample and by amyloid and CDR status. Some data on variables presented are missing and amyloid status may also be missing because CSF biomarker analyses are only available on average 6 months following the baseline visit.

1.1. Cognition and clinical outcomes

The Cognition and Clinical Scientific Advisory Group advised the LCS protocol authors on the construction of the EPAD Cognitive Examination (ECE) (3, 4) as well as on functional outcomes and the capturing of key neuropsychiatric features namely sleep, anxiety and depression. The cognitive outcomes captured are: RBANS (5, 6) (Primary Outcome Measure for EPAD PoC Trial), CDR (7), MMSE (8), NIH Toolbox tests (Dot Counting, Flanker) (9, 10), UCSF Brain Health Assessment (Favorites) (11), Supermarket Trolley Test (12) and Four Mountains Test (13). Function is assessed using the Amsterdam Instrumental Activity of Daily Living Assessment (14, 15). Sleep is assessed using the Pittsburgh Sleep Questionnaire (16); Anxiety is measured using the State/Trait Anxiety Inventory (17) and Depression using the Geriatric Depression Scale (18, 19). Table 1 indicates the ranges for each of these tests and the scores that indicate impairment.

Table 1: Rating scales, their range and direction indicating greater impairment

Test	Range	Direction of Scores Indicating Impairment
RBANS	40-150	Lower
CDR	0, 0.5, 1, 2 and 3	Higher
MMSE	0 - 30	Lower
Dot Counting	0-27	Lower
Flanker	0-10	Lower
Favourites	0-24	Lower
Supermarket Trolley Test	0-28	Lower
Four Mountains Test	0-15	Lower
Amsterdam IADL Assessment	0-80	Lower
Pittsburgh Sleep Questionnaire	0-21	Higher

State/Trait Anxiety Inventory	40-160	Higher
Geriatric Depression Score	0-30	Higher

All cognitive and clinical data is captured on tablets (either on the Medavante Virgil Platform or the UCSF Tabcat System). These data are then uploaded to the EPAD LCS Master Database, held by the EPAD LCS Clinical Research Organisation IQVIA, for conciliation with other data sources e.g. imaging and eCRF data before being quality controlled and then pushed to the Analytical Database hosted by the EPAD Partner Aridhia.

Table 2: Cognition:

	Total Sample (n=500)	**CDR 0 Amyloid – (n=251)**	**CDR 0 Amyloid + (n=118)**	**CDR 0.5 Amyloid – (n=37)**	**CDR 0.5 Amyloid + (n=37)**
RBANS Total (mean/SD)	103.1(12.7)	105.2(12.0)	104.4(10.5)	102.6(14.7)	91.5(14.7)
RBANS DMI (mean/SD)	102.5(13.5)	103.9(11.0)	104.0(12.3)	103.4(14.7)	89.9(19.5)
RBANS List Learning	28.2(4.7)	28.8 (4.2)	29.3(4.2)	27.4(4.6)	23.05(5.4)
RBANS Story Memory	18.19(1.9)	18.5 (2.7)	18.7(2.9)	17.3(3.7)	15.7(4.4)
RBANS Figure Recall	14.2(3.9)	14.5 (3.4)	15.0(3.3)	13.6(3.9)	10.6(4.8)
RBANS Figure Copy	18.6(1.9)	18.6 (2.0)	18.6(1.7)	18.6(2.1)	18.2(1.8)
RBANS Line Orientation	18.0(2.2)	18.0 (2.2)	18.4(1.8)	17.9(3.2)	17.6(2.1)
RBANS Picture Naming	9.8(0.9)	9.8 (0.7)	9.9(0.4)	9.3(2.3)	9.8(0.6)
RBANS Semantic Fluency	19.2(5.6)	20.2 (5.3)	19.4(5.5)	18.9(4.9)	16.4(5.0)
RBANS Digit Span	9.5(2.3)	9.6(2.3)	9.6(2.3)	9.5(1.9)	8.6(2.1)
RBANS Coding	43.9(10.8)	47.0 (9.6)	43.6(9.1)	38.7(13.7)	34(10.7)
MMSE (Mean/SD)	28.6(1.6)	28.7(1.5)	28.8(1.3)	28.6(1.5)	27.6(1.7)

1.2. Neuroimaging Outcomes

The Neuroimaging Scientific Advisory Group advised the LCS protocol authors on structural and functional MRI based evaluations optimised for understanding brain changes in preclinical and prodromal Alzheimer's disease (20). The structural sequences captured in the protocol were Cortical thickness, deep grey matter volumes, fractional anisotropy of temporal lobe, diffusion kurtosis (multi b-valueDTI) and network alterations. The functional MRI outcomes were global & parietal CBF, changes within the default-mode network and relation with hippocampal activity (rsfMRI), Bolus arrival time (multi-delay arterial spin labelling) and network analysis (rsfMRI) although not all of these data have been analysed as yet in V500.0 and therefore not presented in this paper.

All brain-imaging facilities are accredited by the EPAD LCS Imaging CRO IXICO. Imaging files from the site are transferred to IXICO for central reading and safety evaluation. Key outcomes are then transferred to the Master Database for conciliation with other data feeds before these data are transferred to Aridhia and the EPAD Analytical Database. All MRI scanners are 1.5T minimum.

Table 3: Neuroimaging:

	Total Sample (n=500)	**CDR 0 Amyloid – (n=251)**	**CDR 0 Amyloid + (n=118)**	**CDR 0.5 Amyloid – (n=37)**	**CDR 0.5 Amyloid + (n=37)**
MTA Score [Right]					
0	335(67%)	189(75%)	82(69%)	22(59%)	17(46%)
1	117(23%)	56(22%)	31(26%)	7(19%)	17(46%)
2	21(4)	5(2%)	4(3%)	4(11%)	2(5%)
3	7(1.4)	0	1(0.8%)	4(11%)	0
4	0	0	0	0	0
	(n=480)	(n=250)	(n=118)	(n=37)	(n=36)
MTA Score [Left]					
0	344(69%)	207(82%)	77(65%)	24(65%)	14(38%)
1	111(22%)	38(15%)	39(33%)	7(19%)	17(46%)
2	19(4%)	5(2%)	2(2%)	4(11%)	4(11%)
3	5(1%)	0	0	1(2.5%)	1(2.5%)
4	1(0.2%)	0	0	1(2.5%)	1(2.5%)
	(n=480)	(n=250)	(n=118)	(n=37)	(n=37)
Fazeka's Score (Deep White Matter)	129(26%)	80(32%)	29(25%)	8(22%)	6(16%)
0	262(52%)	131(52%)	64(54%)	25(67%)	19(51%)
1	78(16%)	38(15%)	17(14%)	4(11%)	11(30%)
2	11(2%)	1(0.5)	8(7%)	0	0

3	20(4%) (n=480)	1(0.5) (n=250)	0 (n=118)	0 (n=37)	1(3%) (n=37)
Fazeka's Score (Periventricular White Matter	269(54%)	157(62.5%)	61(52%)	23(62%)	12(32%)
0	136(27%)	64(25.5%)	40(34%)	11(30%)	11(30%)
1	66(13%)	27(10.8%)	14(12%)	2(5%)	12(32%)
2	9(2%)	2(0.8%)	3(2%)	1(3%)	1(3%)
3	(n=480)	(n=250)	(n=118)	(n=37)	(n=36)
Hippocampal Volume R (Mean/SD)	2427.9(329.2) (n=275)	2441.3(290.9) (n=247)	2490.5(303.6) (n=117)	2390(322.7) (n=37)	2208(504) (n=37)
Hippocampal Volume L (Mean SD)	2352.9(318.2) (n=275)	2378.4(283.2) (n=247)	2393.7(296.4) (n=117)	2285.7(316.2) (n=37)	2133.1(499) n=37)

	Total Sample (n=500)	**CDR 0 Amyloid – (n=251)**	**CDR 0 Amyloid + (n=118)**	**CDR 0.5 Amyloid – (n=37)**	**CDR 0.5 Amyloid + (n=37)**
Whole Brain Volume	1102848 (108173) (n=474)	1099832 (104810.1) (n=246)	1117058 (108015.9) (n=117)	1095064 (107179.2) (n=37)	1092669 (109142.6) (n=36)
Ventricular Volume	27573.75 (16748.14) (n=475)	23056.92 (12257.53) (n=247)	30952.55 (18485.11) (n=117)	26718.36 (14442.17) (n=37)	39124.83 (19689.35) (n=36)
Pseudo total intracranial volume factor	0.8378453 (0.0903817) (n=475)	0.8259345 (0.0881436) (n=247)	0.8419045 (0.0903157) (n=117)	0.8450905 (0.0886045) (n=37)	0.8722312 (0.0871121) (n=36)
White Matter Lesion Volume	(1.80e+07) (4.17e+07) (n=475)	1.19e+07 3.47e+07 (n=247)	3.27e+07 5.47e+07 (n=117)	5691130 5053915 (n=37)	2.52e+07 5.42e+07 (n=36)

1.3. CSF biomarkers

The Biomarker Scientific Advisory Group, after revision of the existing evidence around neuropathological changes in preclinical and prodromal AD, had to decide which biomarkers were either fully validated as markers of disease or remained at the discovery phase of development. The former were to be incorporated in the protocol whilst the potential to

explore the less validated biomarkers would be reserved for a future date using EPAD LCS samples collected, shipped and stored under optimal conditions. The Biomarker SAG also oversaw the creation of the laboratory manual.

The only biomarkers in the protocol are CSF ABeta, Tau and Phosphorylated Tau. All samples are shipped from the study sites and stored centrally in the EPAD BioBank at the University of Edinburgh before CSF samples, in Sarstedt tubes, are shipped to the University of Gothenburg, Sweden for analysis using the Roche Diagnostics Elecsys Platform. Results are then forwarded to the IQVIA Master Database and then transferred to the Aridhia Analytical Database. Using this system, a threshold of 1,000pg/ml of ABeta42 was agreed upon to define amyloid positivity. The upper limit of analysis for ABeta42 is currently 1,700pg/ml.

Saliva (drooling sample and salivette), urine and plasma are also stored in the EPAD BioBank for future use. To date none of these samples have been analysed.

Table 4: CSF Biomarkers

	Total Sample (n=445)	**CDR 0 Amyloid – (n=251)**	**CDR 0 Amyloid + (n=118)**	**CDR 0.5 Amyloid – (n=37)**	**CDR 0.5 Amyloid + (n=37)**
CSF ABeta	1236.5(426.6)	1486(225.9)	726.4(195.0)	1489.3(254.9)	679.5(164.8)
CSF pTau	19.24(9.7)	18.2(8.0)	18.8(11.5)	18.8(6.3)	26.3(13.9)
CSF TTau	221.8(93.7)	216.7(82.9)	212.6(105.0)	228.0(71.3)	281.6(122.9)
CSF pTau/ABeta Ratio	0.13(0.30)	0.01(0.05)	0.03(0.02)	0.01(0.00)	0.04(0.03)
CSF TTau/ABeta Ratio	0.30(0.29)	0.14(0.06)	0.32(0.18)	0.15(0.05)	0.45(0.28)

In terms of readiness for the EPAD PoC trial, just under the 35% of the V500.0 cohort are amyloid positive with the majority of these being CDR=0 (14.8% of participants with CDR=0.5). To date, 26.6% of the sample have preclinical Alzheimer's disease and 8.3% prodromal Alzheimer's disease. However, with an increasing drive in recent months to significantly increase the proportion of participants with CDR=0.5 to 30%, it is expected that by 2019 and in the commencement of the PoC trial the necessary level of readiness in the LCS will be reached.

1.4. Genetics

The Genetics Scientific Advisory Group had a similar remit to that of the Biomarker SAG in so much as they agreed on recommendations for outcomes to be performed in all samples and within the protocol and advise on optimal storage for future use. They recommended that all participant samples should be tested for ApoE status. Sampling, preparation and storage details can be found in the EPAD lab manual.

Taqman Genotyping was carried out in a single laboratory on QuantStudio12K Flex to establish ApoE variants. Genomic DNA was isolated from whole blood and genotyping was performed in 384 well-plates, using the TaqMan polymerase chain reaction-based method. The final volume PCR reaction was 5 µl using 20 ng of genomic DNA, 2.5 µl of Taqman Master Mix and 0.125µl of 40x Assay By design Genotyping Assay Mix, or 0.25µl of 20x Assay On Demand Genotyping Assay. The cycling parameters were 95° for 10 minutes, followed by forty cycles of denaturation at 92° for 15 seconds and annealing/extension at 60° for 1 minute. PCR plates were then read on ThermoFisher QuantStudio 12K Flex Real Time PCR System instrument with QuantStudio 12K Flex Software or Taqman Genotyper Software v1.3.

Table 5: ApoE Status per CDR and Amyloid PET result:

	Total Sample (n=500)	**CDR 0 Amyloid – (n=251)**	**CDR 0 Amyloid + (n=117)**	**CDR 0.5 Amyloid – (n=37)**	**CDR 0.5 Amyloid + (n=37)**
Age (mean/SD)	66.4(6.7)	64.8(5.9)	65.9(6.5)	69.5(7.6)	71.8(6.6)
Gender					
Female	261(52.2%)	140(54%)	60(23%)	19(7%)	13(5%)
Male	236(47.2%)	111(47 %)	57(24%)	18(8%)	24(10%)
Marital Status					
Married/cohabiting	375	198(59%)	87(26%)	26(7%)	27(8%)
Divorced	54	19(40%)	15(32%)	7(15%)	6(13%)
Single	36	18(60%)	6(20%)	3(10%)	3(10%)
Widowed	32	16(59%)	9(33%)	1(4%)	1(4%)
Years of Education (mean/SD)	14.0(3.7)	14.2(3.6)	13.9(3.8)	13.7(3.7)	14.1(3.9)
Family History (%+)					
Yes	334(67%)	177(71%)	84(71%)	20(54%)	20(54%)
No	166(33%)	74(71%)	34(29%)	17(46%)	17(46%)
ApoE Status					
ApoEe4 Positive (n,%)	190(38%)	91(48%)	59(31%)	6(3%)	21(11%)
ApoEe4/4 (%)	19(10%)	2(11%)	11(58%)	1(5%)	4(21%)
ApoEe4/- (%)	171(90%)	89(52%)	48 (28%)	5(3%)	17(10%)