Conversion prevalence among pre-dementia AD patients & risk factors

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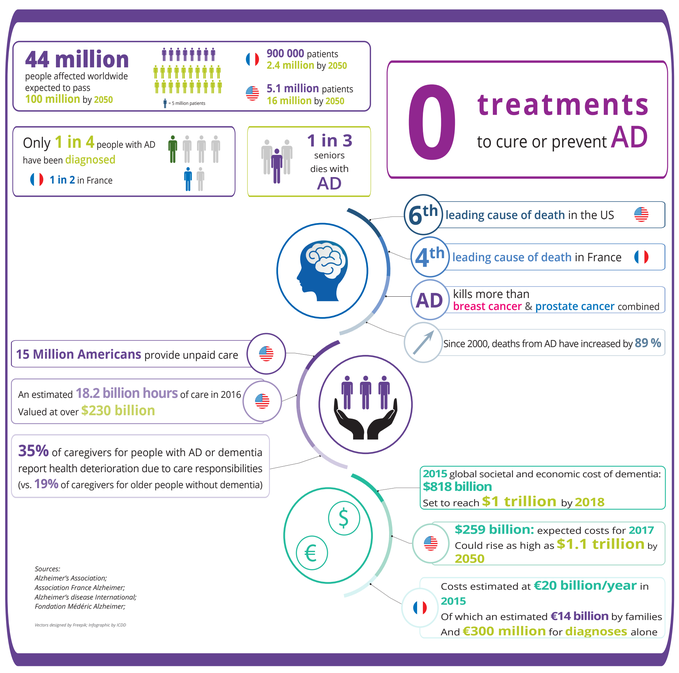
ABSTRACT

- **Background**: The development of disease-modifying therapeutics for Alzheimer's disease (AD) is hampered by the lack of validated biomarkers identifying disease progression at pre-dementia stages. Guidelines for AD diagnosis include markers measuring Amyloid status, Tau protein phosphorylation and neurodegeneration or neuronal injury markers along with the determination of the cognitive decline rate through batteries of neuropsychological tests. Less invasive and more cost-effective tests are needed. ADFlag® is a blood test intending to recognize AD pre-dementia stages. We have previously shown validation results obtained in a multi-cohort, multi-centric setting in Europe. ADFlag® is a scale of 1-5 based on immunologic profiling of circulating blood cells & plasma associated with disease progression as measured by neurological testing including memory assessments, verbal fluency assessments and general cognitive scales. Moreover, in the Pharmacog cohort, the ADFlag® scale correlated with amyloid load in CSF samples. Having an ADFlag® score above 2 at baseline significantly increased the likelihood that conversion to dementia occurred within the 24 months of follow up in the Pharmacog study (OR=4.11, chi sq=0.0030).
- **Methods**: The "Alzpredict" trial and the "Pharmacog" trial patients were used in the present study. The Alzpredict trial is a monocentric ongoing longitudinal trial in which 147 memory-clinic patients were enrolled at baseline. Diagnostics included normal, SCI, MCI and AD patients. The Pharmacog trial is a multi-centric cohort, which enrolled 147 MCI patients at baseline. We measured the ADFlag® score in the Alzpredict and Pharmacog patients. In the Pharmacog cohort, we previously showed that having an ADFlag® score above 2 at baseline significantly increased the likelihood that conversion to dementia occurred within the 24 months of follow up in the Pharmacog study (OR=4.11, chi sq=0.0030). We now used a multivariate regression analysis stratified by age, presence of ApoE4 allele and ADFlag® scale on both cohorts to determine the odd ratio for conversion to AD.

The studies have been approved by the appropriate institutional and/or national research ethics Committees. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

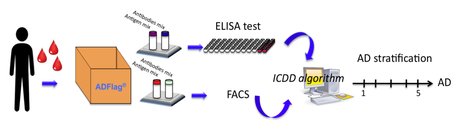
Alzheimer's disease (AD): A major unmet need



Accurate pre-symptomatic diagnostic is crucial

- AD currently lacks appropriate diagnostic tools
  - AD patients are currently diagnosed over the course of 2.5 years, during which disease progression impedes their chances for treatment
  - Markers used for AD diagnosis/prognosis require costly (imaging) and/or invasive procedures (lumbar punctures) and are limited to very specialized centers, increasing costs and barriers to succesful clinical trials (40% of patients will refuse recruitment in a clinical trial because of lumbar punctures (Quintiles))
  - The clinical value of existing tools is questioned by neurologists in the context of the clinical diagnosis of AD with respect to specificity and precision
  - AD clinical practice is very diverse
- Today, recognizing AD prior to loss of cognitive function represents a chance to delay loss of autonomy of AD patients by 2.8 year thanks to existing symptomatic treatments. This would significantly improve patient quality of life and delay the need for institutionalization, saving the health care system 10 billion annually in France alone, making pre-symptomatic diagnostic of utmost importance.
- Despite a past decade marked by setbacks, clinical research is still ongoing and strong, with **143 Phase 2 & 3 clinical trials** recruiting AD patients in 2017
- Pharmaceutical companies lack validated biomarkers for **proper AD patient selection and stratification at pre-dementia stages** to target the disease before irreversible brain damage and mental decline has occured and to identify responding populations
- There is a crucial need for faster, more cost-effective, and more precise diagnostic tools!

ADFlag® measures inflammatory profiles in circulating blood



- Amyloid plaques induce microglia activation
- Recruitment of monocytes at amyloid lesion sites
- Release of pro-inflammatory cytokines from activated microglia & modulation by peripheral immune system
- Peripheral immune system drives microglial proliferation & microvessels lesions
- Chemokines signal microglia activation to proliferation switc

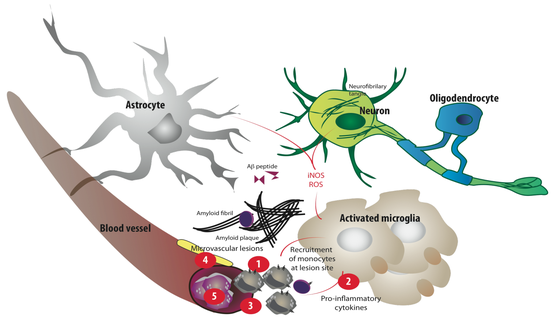
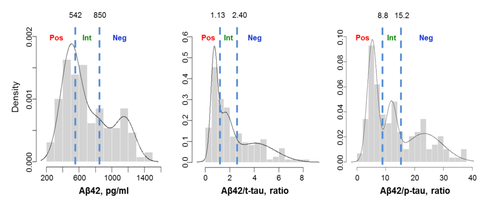


Figure 1: ADFlag® measures inflammatory profiles in circulating blood ; a process associated with AD patophysiology



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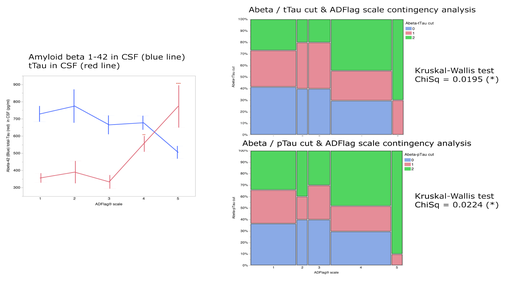
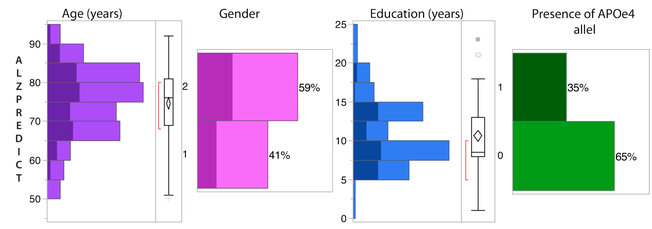


Figure 2: top panel - Distribution of beta amyloid 42 peptides in CSF of patients enrolled in the pharmacog cohort, Botom panel - Amyloid and Tau level in the different ADFlag® score subgroups - Logistic regression between ADFlag® scores and Abeta/Tau and Abeta/pTau scores defining incipient AD.

Two independent clinical trials: screening all patients in the Alzpredict and Pharmacog trials with ADFlag®



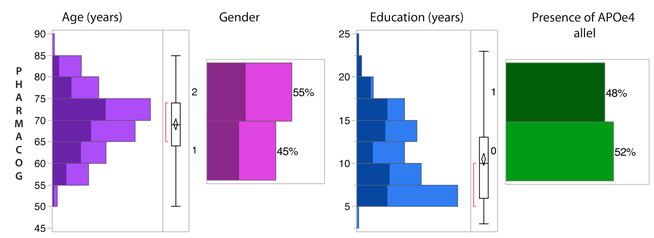


Figure 3: Age, gender, education & presence of APOE4 allel in the patients enrolled in the ALZPREDICT and PHARMACOG trials.

APOE4	non carrier	heterozygotes	homozygous
Alzpredict	62 %	36.33 %	1.67 %
Pharmacog	52.06 %	60.41 %	7.53 %

The probability that a patient carried an APOE4 allel was greater in the pharmacog trial than in the Alzpredict trial (p=0.0338)

Mean Age	Male	Female	p
Alzpredict	77.02+/- 7.83	74.18 +/- 9.59	p=0.006
Pharmacog	69.09 +/-8.08	69.70 +/- 6.83	NS
p	p≤0.0001	p≤0.0001	

In the Alzpredict trial, the enrolled patients were older than in the Pharmacog trial. In the Alzpredict trial, males were older than females; a difference not seen in the Pharmacog trial.

Neuropsychological characteristics			
ADASCog	MMSE	BNT	Delayed RAVLT
10.22+/-9.46	25.00 +/- 5.13	51.20 +/-9.96	6.99 +/- 4.97
12.26 +/-5.08	26.56 +/- 1.78	10.11 +/-0.79	4.01 +/- 3.19
P=0.0136	p=0.0002	p≤0.0001	p≤0.0001

The Alzpredict trial and the Pharmacog trial are two different trials. The Alzpredict trial reflects the «Memory clinic practice»; it included patients with memory complaints (SCI). Mild cognitive impairment patients and early Alzheimer's disease patients. The pharmacog trial included only amnesic MCI patients, with different amyloid beta status.

- Figure 6 shows the distribution of the modeled conversion risk in the different ADFlag® sub-populations
- A normal 2 mixture distribution was seen in all the goups, showing the superposition of 2 normal distributions
- We found that the presence of ApoE4 allel explained the separation in the 2 sub-groups in each sub-population
- Scoring 4 on the ADflag® scale represents the highest conversion risk identified in the model; it is accrued if the patient scoring 4 is also a carrier of the ApoE4 allel.

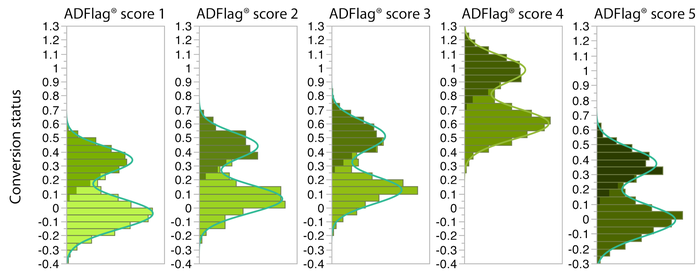


Figure 6: Distribution of conversion risks in the different ADFlag® scores sub-populations.

ADFlag® & conversion to AD

- We hypothesized that if a patient scored 4 or 5 on the ADFlag® scale, they were more likely to convert to AD. Based on this hypothesis, we separated the patient population into patients with high-risk for conversion (ADFlag® score 4 or 5) or with low risk of conversion (ADFlag® score 1, 2 or 3). We then asked whether the high-risk group showed a higher conversion rate.

ADFlag® cut predicts conversion	Converters	Non-converters
ADFlag® high risk group	76.32% (p=0.0012)	48.48%
ADFlag® low risk group	23.68%	43.22%

The probability of not converting is higher when belonging to the low-conversion risk group defined with the ADFlag® scale (p=0.0012).

There are 3.42-fold more chances of conversion if a patient belongs to the high-conversion risk group defined with ADFlag® scale.

Figure 4: Defining risks of conversion based on ADFlag® scoring

To test the relationship between the ADFlag® scores and conversion to AD we ran a nominal logistic regression to better understand if ADFlag® scores could explain conversion. We found:

- That scoring 4 on the ADFlag scale could explain conversion (χ^2 = 0.0081). There was 4.00-fold more chances of converting if scoring 4 than is scoring 1 (χ^2=0.0035). There was 6.36-fold more chances of converting if scoring 4 than if scoring 3 (χ2=0.0030).
- Modeling conversion with ADFlag® scale generated good performances (see fig 5 - ROC) with a PPV of 0.75.
- This model was strengthened by including Age, ApoE4 allel presence and Amyloid beta status as covariates. This increased the performances of ADFlag® scale alone (see fig 5- ROC) and reached a PPV of 0.87.

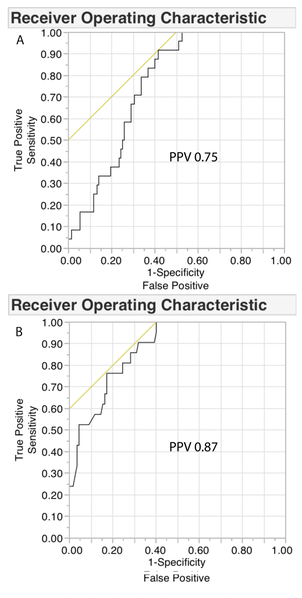
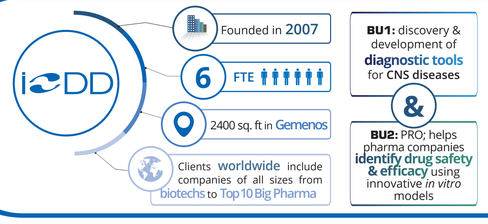


Figure 5: ROC curves showing the classification performances of the two models ; A- using ADFlag® scale as sole classifier, B- Using Age, ApoE4 presence and Abeta status as covariates

CONCLUSION

- 338 patients were analyzed
- Overall, the prevalence of conversion within the two cohorts was 21.53% for a maximal follow-up period of 36 months
- Converters were 73.66 +/-7.99 years of age, similar to the age of non-converters, 50% had an ApoE4 allele (NS), 47.62% were amyloid positive (NS), and 63.16% scored 4 on the ADFlag® scale (p=0.0081)
- We found that scoring 4 on the ADFlag® scale significantly increased the odds for conversion by 4.00-fold compared to patients scoring 1 (p=0.0035) and 6.36-fold compared to patients scoring 3 on the ADFlag® scale (p=0.0030).
- A generalized regression predictive model confirmed the association between conversion into AD and scoring 4 on the ADFlag® scale. The positive predictive value for ADFlag® scores corrected by age, presence of ApoE4 allele and Amyloid beta status was 0.87.
- **Using ADFlag®, a simple, reliable and non-invasive blood test, to screen patients enrolled in preventive or therapeutics AD clinical trials significantly enriches the cohort in incipiend AD patients at pre-dementia stages**
- **Because ADflag® scores 4 and 5 are associated with a positive amyloid-beta status, using ADFlag® can save time and reduce costs in rationalizing patients stratification in AD trials**

About ICDD



BU1: discovery & development of **diagnostic tools** for CNS diseases

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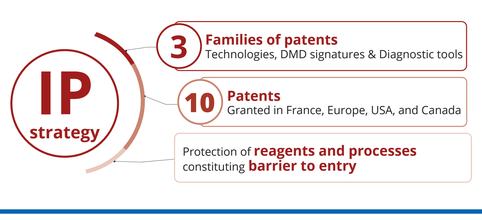
BU2: PRO; helps pharma companies **identify drug safety & efficacy** using innovative *in vitro* models



Blood samples from **700 patients** **banked and annoted** with European standard of care ready to be analysed for **extended validation** of ADFlag®