RDR Challenge
Pre-clinical assay to detect instability of microsatellite repeat expansions

INDUSTRY SPONSORS

- Pfizer

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- Cydan

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AIM
To develop and validate an assay for screening genes and/or compounds that modulate instability of microsatellite repeats. The rarity of repeat expansion/contraction events, estimated to be <1 per 10,000 DNA molecules, creates many challenges for assay development. The goal of this proposal is to devise, implement, and validate an assay that displays the robustness and sensitivity to detect repeat expansion/contraction events after ≤1 week of compound treatment. The assay should utilize a read-out that is suitable for a mid-scale screen of 100s to thousands of compounds in dose response. If such an assay is developed, it will be transferred to Pfizer for further characterization and validation.

BACKGROUND AND RATIONALE
There are >40 diseases caused by expansion of microsatellite repeats. These repeat expansions mediate pathology with a variety of mechanisms including loss of protein expression, toxic aggregation of the transcribed proteins, and toxic gain of function of RNA species that bind essential proteins among others.
There is increasing data supporting somatic expansion of repeats as a driver of disease. A working hypothesis is that in a fraction of cells, the repeats reach a threshold length and are destined to expand further throughout the lifetime of the patient. Because the pathology often correlates with repeat length, cells that have crossed the threshold are irreversibly degenerating.
Because of the stochastic nature of somatic expansion, it may be possible to protect the at-risk population with repeats that have not yet crossed the threshold length. While there are reliable animal models of somatic repeat instability, current cellular models (see Goold et al) require weeks to months of culture and the signal is not robust.

BENEFITS FOR RARE DISEASES
There are >40 diseases caused by expansion of microsatellite repeats. All are genetic (and therefore rare) and degenerative with no disease-modifying therapy. Examples include Huntingon's disease, spinocerebellar ataxias, myotonic dystrophy, Friedreich's ataxia and Fuch's endothelial corneal dystrophy. More conditions are being linked to this mechanism as advances in DNA sequencing technology enable detection of repeat expansions in noncoding DNA. The unmet need is therefore high and increasing as new repeat expansion diseases are discovered. Somatic instability is a shared feature across different repeats and therefore, a therapy that prevents/slows repeat expansion may be able to treat multiple diseases. Drug discovery efforts are currently hindered by the lack of pre-clinical assays monitoring repeat size changes. The understanding of patients' unmet needs in diseases caused by repeat expansions will evolve as the ability to engage with patient communities to gain their perspectives and insights on meaningful benefit as applied to future investigational therapies increases. Consultation with patient representatives is certainly warranted and relevant when we have the ability to modify repeat instability.

HORIZON SCANNING
Data from studies of disease onset and progression in Huntington disease patients and from mouse models of repeat instability have converged on DNA repair as a causal mechanism. The CHDI Foundation has named DNA handling and Repair as one of its seven major focus areas for preclinical research (https://chdifoundation.org/dna-repair-handling/). We are unaware of any additional

initiatives that would fund developing assays of this type. Given the challenges of monitoring this biology, engaging multiple investigators into a consortium is an innovative approach to tackling this problem which would be complementary to the CHDI approach and is an opportunity to bring together stakeholders in several diseases that share a common molecular mechanism.

TIMELINES/MILESTONES AND DELIVERABLES

Stage 1 (M18)

- Assay development.

Identify a readout that can detect repeat expansions/contractions (rare events occurring in less than 1 in 10,000 DNA molecules). Build cellular/biochemical (eg, cell lysates, recombinant proteins, etc) assay that can monitor repeat size changes in ≤ 1 week. The ability to detect expansions in non-dividing cells or cell-free systems is highly desirable. The assay read-out should be based on a technology that is currently available and compatible with medium- to high-throughput screening and the manipulations should be compatible with automation.

Stage 2 (M30)

- Assay validation

Determine whether the performance of the assay is suitable to rank compounds and whether the assay is dependent on known modulators of expansion, eg, known genetic modifiers or tool compounds.

EXPECTED CONTRIBUTION AND EXPERTISE

Expertise would include knowledge of the biology of repeat expansion diseases and experimental methods used to study genomic instability and/or DNA repair at the cellular and/or molecular level. Access to reagents and instrumentation that is compatible with small molecule screening is desirable.

TOTAL BUDGET: 487.500 €

Contribution from the sponsors

In kind

- Sponsors will provide technical input into assay design and requirements.
- Sponsors will be able to contribute with tool compound synthesis.
- If an assay meets the Phase 1 milestone criteria, sponsors will internalize it and evaluate performance, dependence on known modulators of repeat instability, and throughput during Phase 2. The intent is to make the assay, methods, and reagents available to the research community.

Financial

Project Name	Total budget (euros)	N° of industrial partners	Min % cash contribution from industrial partners	Cash contribution from industrial partners included in total budget
Microsatellite repeat expansions	**487.500**	**2**	**30%**	**112.500 (30%)**

Reference

Goold R et al. FAN1 modifies Huntington's disease progression by stabilizing the expanded HTT CAG repeat. Hum Mol Genet.2019 Feb 15:28(4):650-661.