Advanced Cell Modeling: Request for Applications (RFA)

Overview

IDefine, the Kleefstra Syndrome Foundation, seeks to applications that aim to use advanced *in vitro* cellular models to better understand Kleefstra syndrome (KS), and to develop a system that could be used in the future to screen and validate potential KS treatments. Kleefstra syndrome is a neurodevelopmental disorder (NDD) caused by haploinsufficiency of the histone methyltransferase EHMT1. Appropriate methodologies would seek to model the neurological state of the KS patient using patient-derived and isogenic stem cell models, and may include organoids, assembloids, cell printing, or similar cutting-edge technologies. Appropriate readouts may include gene expression, proteomic, morphological, electrophysiological, or other measures to better understand the neurological phenotype of KS brains. The ultimate goal is to improve understanding of the impact of the loss of one copy of EHMT1 on the CNS of patients, and also determine what might constitute "improvement" in these markers when evaluating potential treatments. The funded project will receive $100,000 (USD), with a potential extension of an additional $100,000 in year two. Pre-existing iPSC lines will be provided for use in the funded project.

Background

Kleefstra syndrome (KS) is a rare neurodevelopmental disorder caused by a disruption to one copy of the gene *EHMT1* at 9q34.3 (Kleefstra et al., 2006). This disruption can occur due to pathogenic mutations or structural variants (deletions or rearrangements). The *EHMT1* gene is haploinsufficient, and loss of one functional copy will always result in KS. The disorder is almost always due to a *de novo* genetic event, and has only been known to be carried in families due to mosaicism or balanced translocations in one parent. Therefore, the disorder is evenly distributed throughout the population, and effects all regions and ethnic groups equally. The deletion/mutation in *EHMT1* results in a reduction in active EHMT1 protein, triggering the syndromic features.

KS is characterized by a core clinical phenotype of moderate to severe intellectual disability/developmental delay, autism, and childhood hypotonia. Many KS patients also suffer from epilepsy. Additional risk is present for congenital heart and urogenital defects, behavioral and psychiatric disorders and obesity (Willemsen et al., 2012). A significant number of KS

patients will suffer from severe psychosis/sleep disturbance in adolescence, requiring prompt pharmacologic intervention to stave off a drastic regression in skills (Vermeulen et al., 2017).

Because KS is primarily an NDD, considerable research has been done *in vitro* and *in vivo* to establish the neurological phenotype in model systems. This work has established an *in vitro* electrophysiological phenotype for KS model neurons, as well as multiple downstream dysregulated genes, many of which intersect with other known NDD risk loci (Frega et al., 2019, 2020). Mice with a +/- *EHMT1* genotype have been shown to display a neuroinflammatory phenotype with overactivation of microglia and reduced dendritic spine formation (Yamada et al., 2021). Studies of altered gene expression in mice have also established aberrant patterns of downstream gene expression caused by the reduced dosage of EHMT1 (Iacono et al., 2018).

Scope

Many questions remain about the underlying genotype-phenotype connection in KS in the human CNS:

1) What genes and pathways are over or under expressed due to *EHMT1* haploinsufficiency in humans, and do they differ from what is seen in mouse models?
2) Of these, which are key factors in the observed phenotypes, and which are not critical?
3) What types of neuronal cells are most relevant in the observed phenotype?
4) Is the neuroinflammatory phenotype seen in model mice also present in human brain?
5) What would need to be seen in a model system to indicate "improvement" in these markers?
6) Given the results for items (1-5), are any possible treatment avenues suggested? If so, how should they be screened and validated in the model system in the future?

Eligibility

Principal investigators (PIs) on the application must have a permanent appointment, or be scheduled to begin a permanent appointment by the time of the award. IDefine is interested in seeding additional laboratories that will develop comprehensive programs of research into KS with this award. Therefore, early-career PIs are particularly encouraged to apply. However, applications will be considered from all laboratories capable of delivering the desired research. International applications are welcome, but must be written in English.

Resources to Be Provided

Applications are requested for a grant of $100,000, with a 12 month duration, with an additional extension of $100,000 for an additional 12 months, based on the successful achievement of initially established milestones.

The laboratory selected will be provided with at least two patient-derived and two isogenic *EHMT1*+/- iPSC lines, plus control lines, from IDefine. The proposal should be drafted assuming

the availability of these lines to the research team at no cost. Further, project timelines should assume the immediate availability of the lines at the beginning of the project.

Evaluation Criteria

Applications will be judged on the following criteria:

- The potential of the proposal to provide answers the proposed research questions above
- The potential of the proposal to ultimately provide a system that could be used in future screening studies of potential treatment leads
- The proposal leverages the latest techniques of *in vitro* neurological modeling
- The proposal leverages publicly available data and the latest bioinformatic techniques to maximize the value and interpretation of the experimental results
- Th ability of the research team to deliver the results in the proposed project
- The potential of the proposal to lead to an ongoing KS research program in the selected laboratory

How to Apply

Submission of all questions, correspondence, and materials should be made via email to grants@idefine.org.

Phase1: Letter of Intent (LOI)

Interested researchers meeting the eligibility should draft an LOI of up to three pages. It should include:

- A high-level description of the proposed project including specific aims
- A description of how the project will address the goals described in this notice
- A description of what future directions would be opened up by the proposed work
- A statement on the resources, capabilities, and personnel available to the lab, and how they will allow the work to be executed at a cutting-edge level
- A CV for the project PI (not counted in the three-page limit)

Early submissions of LOI are highly encouraged, see schedule below.

Phase 2: Full Grant Proposal

Two to four applicants will be selected to submit a full grant proposal based on evaluation of the LOI. The full grant proposal should include:

- A scientific abstract of the proposed project
- A full scientific background for the proposed approaches and how they will fit in to the goals outlined above, and the previous research done in the KS field
- An expanded project description and specific aims
- Preliminary data for the approaches to be used (if applicable)

- A detailed research plan for the work including a timeline of key milestones for each phase, and when they are expected to be achieved
- A list of how funds will be used in the proposed project, priced in US dollars (Note: as part of a board-directed policy, IDefine will not pay for any indirect costs out of grant funds)
- A discussion of expected outcomes and knowledge that the work may generate
- A discussion of potential pitfalls, and how they will be addressed if encountered
- A discussion of potential future directions for the work beyond the duration of the grant, including the expected applicability of the work in future screening/discovery of potential treatment leads for KS

Initiation of Funding

The successful applicant will receive the initial funding and materials outlined above immediately, contingent on the successful negotiation of an acceptable research contract with IDefine. Funding for year 2 will be contingent upon accomplishment of the agreed milestones, and at the sole discretion of IDefine.

Application Schedule

The following schedule will be followed:

Release of this RFA	Mar 1, 2024
Due date for letters of intent (LOI) sent to grants@idefine.org	April 15, 2024
Invitation to applicants for full proposal based on LOI	May 15, 2024
Full proposal due	July 15, 2024
Notification of winning applicant	Sep 1, 2024

References

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