ITP proposal: MIF098 (MIF antagonist)

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Rationale. MIF (macrophage migration inhibition factor) is a widely expressed and pleiotropic cytokine with immunoregulatory, neuroendocrine, and metabolic actions (1). MIF expression is required for optimal pro-inflammatory responses and MIF has a close regulatory interaction with glucocorticoids by virtue of its ability to specifically suppress their anti-inflammatory function (4,5). MIF promotes tumor progression by several mechanisms and is expressed in elevated levels in many invasive and metastatic cancers (6,7). MIF also is a strong inducer of AMP-activated protein kinase (AMPK) and promotes glucose uptake and energy utilization in hypoxic cells (8). It is further notable that MIF is encoded in a functionally polymorphic locus within the human genome (22q11.23). Variant *MIF* alleles occur commonly in the human population (minor allele frequency >5%) and high expression alleles have been linked to the clinical severity of a number of autoimmune inflammatory, infectious, and oncologic diseases (9), suggesting that pharmacologic reduction of MIF activity could extend healthy, disease-free life.

That MIF expression may influence longevity was suggested initially by studies of caloric restriction (CR), where it was reported that mice maintained on either of two anti-aging diets, CR or a diet low in methionine (Meth-R), had significantly elevated levels of MIF mRNA in the liver (10,11). An influence of MIF on lifespan was addressed directly in a recent study of MIF-KO mice (B6x129 genetic background) fed *ad libitum* (AL) or a CR diet (2). Median survival in the AL control mice was 774 days while median survival in the AL MIF-KO mice was 16% higher (895 days) (Fig. 1). Among CR mice, median survival was 1045 days and was 1064 days in the CR MIF-KO, which is an increase of 35 and 19%, respectively, relative to AL mice. Notably, not only did MIF-KO mice show a lifespan extension in response to CR, they were longer lived than controls under standard AL feeding conditions. Another lifespan experiment, using a different genetic stock with much longer control lifespan, is now in progress but does not so far show a lifespan benefit (R. Miller, *unpublished*). Additional work will be needed to establish whether genetic or pharmacological modulation of MIF action will lead to improved healthspan and lifespan in mice.

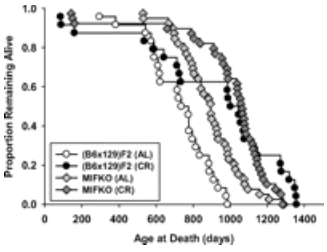

Fig. 1. MIF-KO mice are significantly long lived (log-rank, $P<0.001$) relative to (B6×129)F2 control mice, and also exhibit a significant life-span extension in response to CR. Kaplan-Meier survival curves for each genotype and dietary condition; each point represents a single mouse. From (2).

MIF Antagonism. Immunoneutralization or genetic deletion of MIF reduces disease severity in virtually every pre-clinical model of inflammatory disease that has been studied, prompting the development of pharmacologic MIF inhibitors for clinical application (9,12). An anti-MIF monoclonal antibody developed by the Yale co-sponsor has been humanized for phase I clinical testing for lupus nephritis and for solid tumors (13,14) (*ClinicalTrials.gov Identifier*: NCT01765790). Potent and pharmacologically auspicious small molecule MIF antagonists that block MIF interaction with its receptor also have been developed by the Yale laboratory (15). One such antagonist: MIF098 (3-(3-hydroxybenzyl)-5-methylbenzo[*d*]oxazol-2(3*H*)-one) is orally bioavailable and well-tolerated in mice, and shows MIF inhibitory activity in mouse models of bronchopulmonary dysplasia, hyperoxic lung injury, and in an OLAW approved collagen-induced model of arthritis (3,16-18) (Figs. 2,3).

Further noteworthy for this proposal are observations that dietary isothiocyanates, such as sulforaphane present in cruciferous vegetables, inactivate MIF by binding covalently to the same domain employed in the structure-based design of MIF098 (19). In a controlled study, subjects ingesting sulforaphane were demonstrated to have reduced MIF activity in urine (20). There is much current interest in the use of sulforaphane to attenuate the severity of oxidant, electrophile, and inflammatory stresses that contribute to the pathogenesis of many chronic diseases including aging (18-20), and it is possible that some of the benefits of sulforaphane or other Nrf2 inducers may be attributable to direct inhibition of MIF function.

The sponsors are currently unaware of any studies planning to address the impact of MIF antagonists on longevity. Because MIF098 emerged from a rational, structure-based design program, shows specificity for the MIF-MIF receptor interaction, and has undergone favorable toxicologic and pharmacokinetic testing in rodents, we propose to utilize it in a study of mouse lifespan extension.

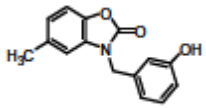

Fig. 2. Chemical structure of MIF098, 3-(3-hydroxybenzyl)-5-methylbenzo[*d*]oxazol-2(3*H*)-one, identified by structure-based molecular design to inhibit the interaction between MIF and its cell-surface receptor, CD74 (3).

Activity, Dosage, Bioavailability, and Toxicity. MIF098 reduces the K_D for MIF binding to its receptor by 5.1 fold (from K_D = 6.5 x 10^{-9} to 3.3 x 10^{-8} M) and shows superior antagonism of MIF-dependent ERK1/2 phosphorylation when compared to previously described, prototypic MIF inhibitors. In a cell-based signal transduction assays, MIF098 reduces ERK1/2 phosphorylation by 200-fold when compared to the inhibitor ISO-1 (3), a structurally related MIF inhibitor previously shown to protect mice from the severe inflammatory complications of infection or autoimmunity (13,21). MIF098 recapitulates *Mif*-gene deficiency in established mouse models of broncho-pulmonary dysplasia (17) and hyperoxic lung injury (18). We also have recently completed a study of MIF098 in collagen-induced arthritis, and the drug showed equipotency with prednisolone (dosed at 3 mg/kg qd) and was well-tolerated over the 20 day course of daily therapy (dosed at 20 mg/kg ip bid). A small but significant measure of improved efficacy was observed by dosing MIF098 at 40 mg/kg bid or 80 mg/kg qd. Importantly, equivalent therapeutic efficacy was observed by parenteral (ip) as by oral (gavage) dosing (*data available upon request*).

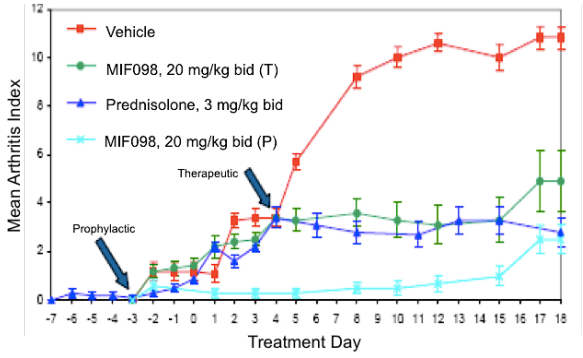

Fig. 3. Efficacy of MIF098 in the collagen-induced arthritis sensitive DBA1/J mouse strain. Mice were immunized with bovine collagen prior to treatment in either a prophylactic (before disease induction) or therapeutic (after disease onset) protocol.

There is currently no experience with administering MIF098 in food or water, but no special precautions are considered necessary to ensure compound stability. Pilot studies could quickly determine the level of serum MIF098 in UM-HET3 mice given food containing MIF098 at plausible levels, *e.g.* at 40 mg per kg body weight per day, with dose adjustment if needed prior to the initiation of the longevity study. MIF098 is stable and should maintain full activity in food held at zero degrees for many months.

We note that three of the agents found by the ITP to prolong mouse lifespan have immunosuppressive and/or anti-inflammatory activity, including rapamycin, aspirin, and NDGA. MIF, by opposing the anti-

inflammatory activity of glucocorticoids, is considered pro-inflammatory, and it seems plausible that some of the benefits of MIF inhibitors on illnesses reflect blunted inflammatory responses. The recent report that targeted down-regulation of NFκB pathways in mouse hypothalamus can extend mouse lifespan provides additional justification for testing drugs that blunt inflammatory tone in multiple organ systems (22).

We would propose to start the agent at 4 months of age, and continue lifelong administration. There are no validated biomarkers of quantitative measures of pharmacological efficacy of the MIF098 inhibitor in whole mice, however we will test for MIF's intrinsic tautomerase activity in treated mice following the protocol that showed the impact of sulphorane ingestion (20).

Cost of a Life-long Intervention Study. Initial quantities of MIF098 (GMP grade) are available from Yale or may be readily synthesized by a third party CRO at reasonable cost ($64 per gm). A study that administers MIF098 at 40 mg/kg qd would require purchase of food at 240 mg of MIF098 per kg of food. (Each mouse would receive 1.2 mg of MIF098 daily). A typical ITP protocol uses 1300 kg of food for the three sites from age 4 months to death. At 240 mg per kg food, this protocol would require a total of 312 grams of MIF098. At a cost of $64 per gram, total cost for the agent would be $19,968 for the entire study, or approximately $7000 per year from 4 months of age onward.

In submitting this proposal, we agree to the following:

We understand all information presented in the proposal can be freely shared with members of the ITP Steering Committee and Access Panel during their evaluation of proposals, but will otherwise be considered confidential.

If our proposal, or a modification of it (such as altered dosage or frequency of administration), is accepted for inclusion in a research protocol, we will be asked to help evaluate the data and to prepare the data for written and oral publications, on each of which we will be offered coauthorship. We understand the ITP intends to submit the results of all ITP-supported studies—regardless if they produce data showing positive or negative effects on health status in mice—for publication.

We understand data generated by ITP-supported experiments using the compound/diet proposed will be made publicly available and can be used in applications for further research support by anyone. We also will be free to use ITP-generated data in the context of applications for research support or for any other purpose.

The compound/diet proposed makes use of materials that are not yet freely available and whose production depends on proprietary or unpublished methods. If our application is approved for incorporation in the ITP, a mutually acceptable Materials Transfer Agreement that would permit us to provide the ITP with the compound(s) needed for the experimentation will be developed with the institutions involved in this program.

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