Project Marilyn Proposal

Summary

Project Marilyn is a nonprofit venture to raise money using the crowdfunding mechanism to bring several anticancer drug candidates through preclinical trials. Unlike most pharmaceutical research, the candidates brought forward by PM **will not be patented** and instead released to the public domain, so that ultimately manufacturers can produce these candidates inexpensively without licencing costs. The project lead, Isaac Yonemoto, was involved in producing and conducting initial tests on the parent compound, 9-deoxysibiromycin (9DS).

Background

Sibiromycin was discovered in the last century by researchers in Siberia. It is a toxic compound produced by soil bacteria and functions by arresting DNA replication. Therefore, it has potential as an anticancer chemotherapeutic. Early research on sibiromycin was abandoned when it was discovered to also be cardiotoxic. Later research on the family of chemicals identified a single oxygen atom that is likely to be the cause of the undesirable cardiotoxicity. Dr. Barbara Gerratana devised a method to produce analogs of sibiromycin lacking this oxygen atom, hence 9-deoxysibiromycin. Initial tests indicate that as designed, 9DS is **less cardiotoxic** than the parent sibiromycin, and surprisingly, **more potent** against cancer cell lines *in vitro*. Recently, a related compound, SJG-136, has met some success in initial clinical trials.

Dr. Gerratana departed from her position as a primary investigator, and once again research on the sibiromycin family was abandoned. Project Marilyn picks up where this left off, taking advantage of the expiration of the patentability of 9DS and continuing with the intent of producing more analogs and releasing these to the public domain as well. An open-access version of the publication reporting 9DS is available.

What remains to be done is to bring 9DS through further preclinical experimentation, including animal studies. A pharmacokinetics study and a xenograft study will evaluate suggested dosage and measure efficacy against select cancer lines *in vivo*. Pharmaceutical development is often ‘a numbers game’; to further improve the likelihood of success, PM seeks to produce analogs of 9DS for preclinical evaluation, so that ultimately ‘best candidates’ can be put forth for clinical trials. The method developed by Dr. Gerratana is particularly amenable to the production of analogs.

Project Lead Qualifications

Isaac Yonemoto earned his PhD in chemistry from The Scripps Research Institute, for research on protein folding dynamics. Subsequently, he performed postdoctoral research under the supervision of Barbara Gerratana, gaining firsthand experience in the production, analytical verification, and handling of sibiromycin and analogs. After this, he performed postdoctoral research at the J. Craig Venter Institute, gaining experience in synthetic biology techniques. A full CV is available here.

Fundraising

Fundraising will begin on January 7th, 2014 and last till February 7th, 2014, not inclusive of extensions. To improve the likelihood of success (including a small budgetary margin), the fundraising minimum "tilt value" will be 750,000 USD. A first stretch goal will be announced and described at 1,000,000 USD and will tilt at 1,250,000 USD. A second stretch goal will be announced and described at 1,500,000 USD and will tilt at 1,750,000 USD. If enough funds are collected to reveal a stretch goal (i.e. at the 1,000,000 and 1,500,000 levels), then the fundraising period will be extended one week (i.e. to February 14th, and February 21st, respectively). Although we will not 'reveal' the stretch goals at the moment, both stretch goals will be projects dedicated to the treatment of cancer, and will be well-posed to share laboratory infrastructure with the primary PM objective. All pharmaceutical candidates developed for the primary or stretch goals will not be patented and will be released to the public domain.

Success Criteria

PM will be considered to have succeded if three sibiromycin derivatives (including 9DS) have completed a suite of preclinical trials, to include NCI-60 screen, pharmacokinetic studies and xenograft studies.

Secondary Objectives

PM will also pursue some secondary objectives which will not affect the determination of success but may in the short term ease the production of sibiromycin analogs for preclinical testing and in the long term improve the likelihood of regulatory approval or commercial viability. These include, but are not limited to: development of producer strains, improvement of growth and harvesting conditions for producer strains, and improvement of purification process.

Budget Summary

Budgets for 750,000 USD, 1,250,000 USD, and 1,750,000 USD are presented. Note that higher fundraising will result in increased budgetary margins. Also note that the 1,750,000 USD stretch goals involves experimentation over a longer timeframe than the other two fundraising outcomes. Employees will not be penalized if the project concludes earlier as a result of achieving the project objectives.

Surplus Budget

Upon conclusion of the project, the use of the surplus budget will depend on the total amount of funds raised by crowdfunding:

- → 750,000~1,000,000 USD, the surplus budget will be transferred to a 501(c)(3) nonprofit cancer research hospital, such as City of Hope hospital.
- → 1,000,000~1,250,000 USD, no more than half of the surplus budget will be used to pilot the first stretch goal with the intent to generate data to re-pitch the first stretch goal pending the success of PM primary objective. The remainder will be transferred to a 501(c)(3) nonprofit cancer research hospital.
- → 1,250,000~1,500,000 USD, the surplus budget will be transferred to a 501(c)(3) nonprofit cancer research hospital.
- → > 1,500,000 USD no more than half of the surplus budget will be used to pilot or continue the first and second stretch goals. The remainder will be transferred to a 501(c)(3) nonprofit cancer research hospital.

10% time

The freedom to pursue tangential or unrelated science can yield important insights for the main project. Postdoctoral researchers (but not technicians, without approval of the project lead) will be encouraged to pursue “10% time” projects which may or may not be related to PM, if funds are available. Funds for reagents, equipment, and supplies for 10% time will derive from the indysci general budget, and not the PM budget, unless a compelling connection to PM can be argued. The project lead will be responsible for making this determination. Salaries for 10% time will not be prorated.

Other Projects

The project lead will reserve the right to pursue at most one other primary scientific project outside of the scope of PM and the PM stretch goals. If such a project is funded, then the lead salary will be prorated to 50%.

Attachments

- → Full Scientific Description
- → Budget spreadsheets
- → Timeline
- → Lead Researcher CV

Project Marilyn

Official Research proposal

Isaac T. Yonemoto

Executive Summary

I propose experiments to further the development of the sibiromycin anticancer drug family. 9-deoxysibiromycin (9DS) was developed in the Gerratana lab as a novel improvement over the natural product sibiromycin. While designed to be less cardiotoxic than its parent compound, *in vitro* experiments strongly suggest that it is both **less cardiotoxic and more powerful**. Here I describe three experiments: an analysis of the DNA-binding specificity of sibiromycins, diversification of the molecule, and refactoring the production platform. Overall, the goal is to conduct basic preclinical science and biochemical engineering to improve the likelihood of clinical success at curing cancer.

SIGNIFICANCE

As a heterogeneous class of conditions, cancer is a disease which is therapeutically challenging. Each patient has a highly individualized disease, whose etiology and outcome is dependent on underlying genetics, environment, and stochastic factors. Therefore broad-spectrum therapeutics target a few properties common to all cancers. Historically, the most celebrated broad-spectrum agents prevent cancer cell replication by attacking DNA itself or critical replication events. The most successful of these therapeutics have been natural-products and natural-product-based compounds, probably because they arise from evolutionary pressure to develop cellular warfare agents.

This proposal focuses on a class of natural products called pyrrolobenzodiazepines (PBDs). PBD cytotoxicity results after the PBD alkylates the exocyclic nitrogen of guanine bases in DNA [1,2]. As a class, PBDs do not appear to cause bone marrow suppression, which is a major side effect of most chemotherapeutic agents that attack DNA [3]. Of the known PBDs, the most potently cytotoxic is sibiromycin, a compound biosynthesized by the actinomycete *Streptosporangium sibiricum* [4]. Clinical research was not pursued because it was discovered that the related compound anthramycin was cardiotoxic [5]. Other members of the PBD molecule class have since been identified, and it appears that cardiotoxicity correlates with hydroxylation at the 9-position of the molecule [6]. If removing the hydroxylation could result in a compound that were less cardiotoxic molecule, the properties of PBDs would make Sibiromycin an attractive pharmaceutical lead.

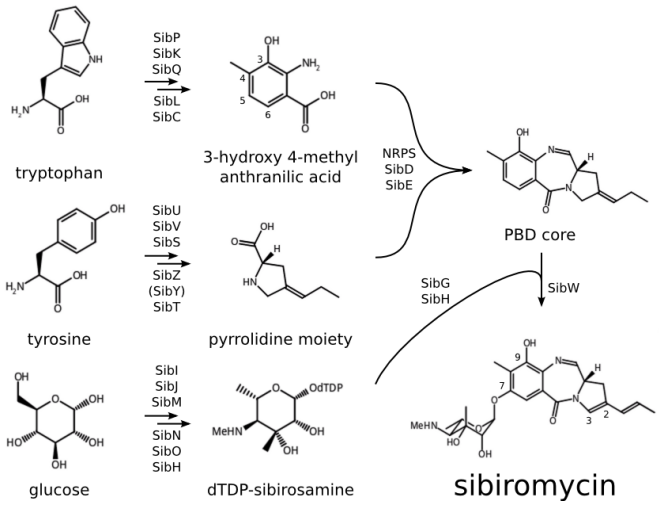

Figure 1: Sibiromycin biosynthesis is convergent. A strategic knockout in the anthranilic acid arm (top row) allows modified anthranilic acids to substitute in the benzyl ring of sibiromycin.

Recently, Barbara Gerratana and coworkers identified the sibiromycin biosynthetic cluster with the aim of modifying the biosynthesis to produce the 9-deoxy congener of sibiromycin [7]. This was achieved by deleting a key gene in the convergent biosynthesis and feeding a chemical precursor that lacks the oxygen analogous to the 9-oxygen in sibiromycin. My previous work with Dr. Gerratana was to isolate, scaleup, and chemically characterize sibiromycin and 9-deoxysibiromycin (9DS) [8]. Biological characterization of 9DS revealed that it is approximately ten-fold less cardiotoxic and five fold more cytotoxic than the parent sibiromycin compound. GI_{50} values are in the low nanomolar concentrations, a significant increase in the therapeutic window of these compounds.

The experiments proposed here move forward development of drugs based on the sibiromycin scaffold. This is pharmaceutically significant because of the validated toxicity profile of PBD drugs, because of the high potency of sibiromycin, especially 9DS, and because it appears we have ameliorated the most serious roadblock to sibiromycin development, its cardiotoxicity.

EXPERIMENTAL SUMMARY

Major objectives

Diversify the PBD scaffold of sibiromycin

Produce 9-deoxysibiromycin in a production scale organism

Preclinical evaluation of sibiromycin analogs

Minor objectives

Develop improved isolation and purification of sibiromycin

Develop efficient synthesis of anthranilic acid analogs

MAJOR OBJECTIVES

Diversify the PBD Scaffold of Sibiromycin Although the therapeutic window for 9DS appears to be favorable, the limited *in vitro* experiments we have performed so far are no guarantee that it will exhibit adequate performance when translated the clinic. *De novo* determination of factors influencing therapeutic success, including potency, toxicity, bioavailability, and pharmacokinetics, is currently and for the forseeable future infeasible. Medicinal chemistry remains largely an empirical art. For a novel scaffold like sibiromycin, a structure-to-activity relationships (SAR) profile of variants can reveal insight on how to optimize the molecule's biological properties.

Traditionally, medicinal chemists employ the process of diversification to increase the odds that at least one candidate in the compound family will be clinically effective and pass the regulatory gauntlet. Even natural products, which enjoy a much higher rate of success, are often subjected to medchem optimization. Luckily, sibiromycin biosynthesis is convergent. Mutasynthesis has already produced the 9-deoxy analog. Further analogs may be produced as a direct extension of the previously achieved process.

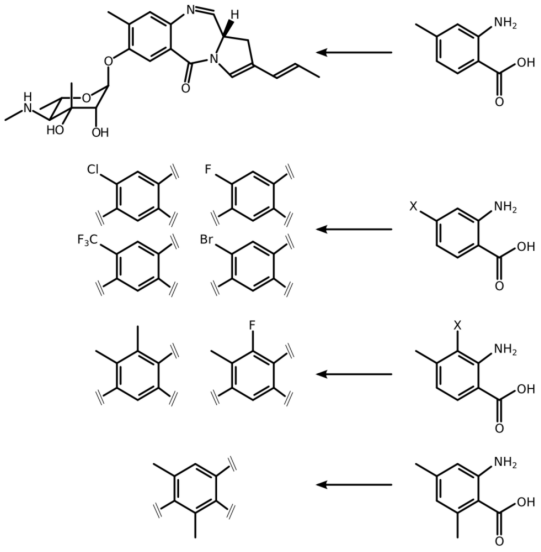

Figure 2: Example 9-deoxysibiromycin analogs that may be produced by mutasynthesis.

Challenge: Produce at least three analogs of sibiromycin building upon the scaffold of 9-deoxysibiromycin.

Method: A series of commercially available or easily-synthesized anthranilic acids will be fed to the modified *S. sibiricum* strain. These anthranilic acids should cause corresponding modifications to the PBD ring A system. In some cases the adenylation domain of SibD (the enzyme unit responsible for anthanilic acid activation) may need to be altered to allow the enzyme to accept the substituted anthranilic acid. In those cases, structural homology to solved adenylation domains can be used to guide our choice of mutations.

Success criterion: This objective will be considered to have succeeded if three analogs of sibiromycin are produced, purified, and characterized by NMR.

Potential pitfalls:

- Insufficient yields of analogs
- Inability of enzyme to accept the desired analog (enzyme might depend on substituent at the varied position)
- Inability of organism to uptake analog precursor
- Inability of efflux pump to export the relevant analogs, killing the S. sibiricum strain

Produce 9-deoxysibiromycin in a production scale organism The strain producing sibiromycin is an uncharacterized *Strepospo*rangium species. The uncharacterized nature of this strain may cause regulatory difficulties leading to approval. The purification technique I developed to isolate sibiromycin and 9DS was mostly chromatographic in nature. Unlike recrystallization, achieving ultra-pure formulations by chromatography is difficult. In such a situation, it is important to have assurance that the panel of secondary metabolites that may come through in the chromatographic separations do not cause batch-to-batch variations in potency or safety. Species producing drugs approved for medical use include *S. cerevisiae*, *S. pombe, E. coli*, and *Streptomyces avermitilis*. Moving to a more well-defined strain even if not pre-approved, would also lend itself more easily to downstream optimization of production yield and reduction of side products by using synthetic biology methods.

Challenge: Create a microbial strain that is capable of producing sibiromycin analogs.

Method: Use PCR and Gibson isothermal assembly to create a refactored sibiromycin biosynthesis cluster. Manipulation of large DNA is difficult but I have experience with the requisite techniques. Briefly, genes will be grouped by structural function and integrated into a single, unidirectional operon, driven by a promoter either from *S. griseus* or *Mycobacterium smegmatis*. At various stages of assembly the biological function of each sub-assembly can be tested. For example, after the resistance gene is cloned, cell viability in the presence of antibiotic will be tested; after the NRPS modules are added, extracts will be tested for antibiotic activity as an indicator of the presence of sibiromycin aglycone. Other tests may include harvesting, chromatographic repurification and spectroscopy (such as NMR) to identify the expected metabolite.

Success criterion: This objective will be considered to have succeeded if either a strain of *Streptomyces* or *M. smegmatis* are engineered to produce 9-deoxysibiromycin at a per culture volume titer at least one tenth that of *S. sibiricum*

Potential pitfalls:

- Inability to transform host cells with large DNA constructs
- Acute toxicity or failure of resistance mechanism in host cell
- Promoter incompatibility or transcriptional failure
- Failure to detect biosynthesized protein

Preclinical evaluation of sibiromycin analogs The ultimate goal of Project Marilyn is to complete preclinical studies on as many sibiromycin analogs as possible - to provide the public a set of 'ready targets' for further development as clinical entities. While much of the internal work is to finish the process of developing new molecules for study, the next step is to begin the process of evaluating these molecules in biological settings.

Method: Initial screening of anticancer potential for small molecules is routinely performed by the National Cancer Institute (NCI). Briefly, the NCI curates a panel of 60 patient-derived cancer cell lines which are considered to be ideotypical of human cancers in general. Small molecules delivered to the NCI are administered to the cell lines and inhibition of growth (GI50) is measured, as a first estimation of the molecule potency. Comparison of activity across different cell lines can provide a qualitative sense of the activity spectrum and insight into mechanistic details. Sibiromycin and 9-deoxysibiromycin have both been subjected to testing against this cancer panel.

Preclinical screening of anticancer drug leads then proceeds to in vivo animal models. Two experiments are of particular relevance: pharmacokinetics ("PK") and xenograft ("XG") studies. PK studies establish the persistence and stability of the drug in the animal system, to give a reasonable range of drug dosage and begin the process of cataloging side effects of drug administration. XG studies are the in vivo analog of the NCI panel; mice are implanted with human cancer cell lines, treated with varying dosages of the drug and the efficacy of the drug is measured. These experiments can also be considered to be roughly analogous to the human Phase 0/I and Phase II/III trials as defined by the FDA. These experiments are typically conducted by contract research organizations (CROs) who have aggregated expertise and can take advantage of economies of scale in animal maintenance and analysis.

Success criterion: This objective will be considered to have succeeded if three compounds, 9-deoxy-sibiromycin inclusive, have been fully assessed in the NCI60 panel and in a PK and XG study in an animal model.

Potential pitfalls: Note that this sub-project will be conducted by public and private contract research organizations (CROs), so there are few pitfalls that would be in the hands of indysci.org

- produced compound is insufficient to complete PK or XG studies
- produced compound contains impurities which complicate analysis

MINOR OBJECTIVES The minor objectives of Project Marilyn are aimed toward improving the economy and efficiency of sibiromycin and sibiromycin analog production. Although the success of Project Marilyn overall is not incumbent on their individual successes, successes would facilitate the primary objectives, expand the scope and potential of the discoveries made by Project Marilyn, or improve access to sibiromycin in the future.

Develop improved isolation and purification of sibiromycin Because of the reactivity profile of sibiromycin, the molecule is sensitive to both high and low pH conditions, and also may exhibit a moderate sensitivity to exposure to light. Further, the producer S. sibiricum strain appears to produce molecules with slight structural variation to sibiromycin, compounded by difficulties caused by the equilibrium population of two diastereomers in alcoholic solutions. An ideal isolation would feature recrystallization of sibiromycin - affording reproducibly high purity with minimal handling. Several strategies to effect a recrystallization can be tested, for example modification of the reactive imine by reversible protection as a phenolamine.

Develop efficient synthesis of anthranilic acid analogs Anthranilic acids are notoriously difficult to synthesize and purify partially due to their limited solubility in typical organic solvents and water. Furthermore, not all anthanilic acids that we would like to test are available from commercial sources. Thus, development of better syntheses will be of interest to further Project Marilyn's goals. Recently, high yield syntheses from inexpensive anilines have been reported featuring advances made in the new field of C-H bond activation. Ideally, the protecting group required for this chemistry can be left on the anthranilic acid and enzymatically removed by the producer bacterial strain, requiring a single step and minimal preparation to go from inexpensive commercial product (aniline) to feeding the bacteria and generating the sibiromycin analog.

References

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Funds:	750,000	Categories	Employment			Physical Overhead	Recurring Costs			Research Infrastructure		CRO/CRS				Other
Date	Running Total	Month Total	Lead Researcher	Postdoc	Technician	Rent + Utilities	Reagents	Supply	NMR Budget	Fume Hood	Apparatus	DNA	sequencing	PK	XG	publications
1/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3/1/2014	166,375	166,375	4,375	0	0	3,000	3,000	1,000	0	100,000	40,000	15,000	0	0	0	0
4/1/2014	187,400	21,025	4,375	4,700	4,700	3,000	3,000	1,000	0	0	0	50	200	0	0	0
5/1/2014	208,425	21,025	4,375	4,700	4,700	3,000	3,000	1,000	0	0	0	50	200	0	0	0
6/1/2014	229,750	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
7/1/2014	251,075	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
8/1/2014	272,400	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
9/1/2014	293,725	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
10/1/2014	315,050	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
11/1/2014	336,375	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
12/1/2014	357,700	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
1/1/2015	379,025	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
2/1/2015	400,350	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
3/1/2015	421,675	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
4/1/2015	443,000	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
5/1/2015	464,325	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
6/1/2015	485,650	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
7/1/2015	506,975	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
8/1/2015	528,300	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
9/1/2015	549,625	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
10/1/2015	570,950	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
11/1/2015	592,275	21,325	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	50	200	0	0	0
12/1/2015	613,350	21,075	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	0	0	0	0	0
1/1/2016	634,425	21,075	4,375	4,700	4,700	3,000	3,000	1,000	300	0	0	0	0	0	0	0
2/1/2016	655,200	20,775	4,375	4,700	4,700	3,000	3,000	1,000	0	0	0	0	0	0	0	0
3/1/2016	749,975	94,775	4,375	4,700	4,700	3,000	0	0	0	0	0	0	0	36,000	36,000	6,000
Totals	749,975	749,975	109,375	112,800	112,800	75,000	72,000	24,000	6,000	100,000	40,000	16,000	4,000	36,000	36,000	6,000
Percent	100%	100%	15%	15%	15%	10%	10%	3%	1%	13%	5%	2%	1%	5%	5%	1%
category total			45%			10%	14%			19%		12%				1%

Employmer	45%
Physical Overhead	10%
Recurring Costs	14%
Research Infrastructu	19%
CRO/CRS	12%
Other	1%
margin	0%

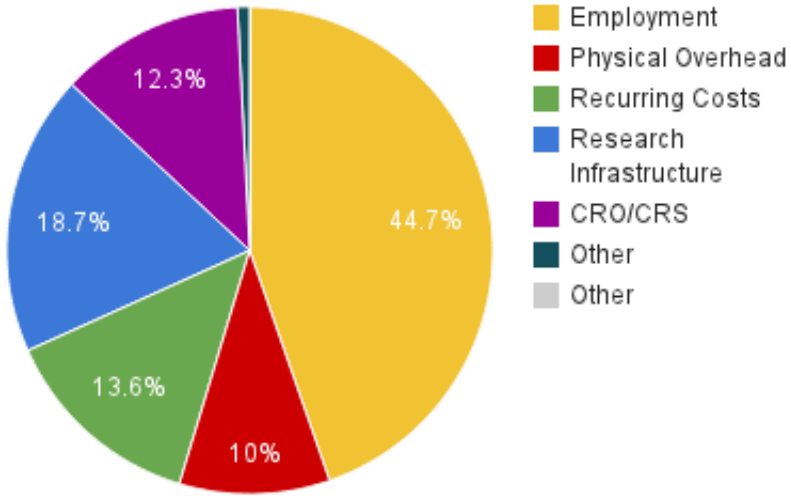
Funds:	1,250,000	Categories	Employment				Physical Overhead	Recurring Costs		Research Infrastructure				CRO/CRS				Other
Date	Running Total	Month Total	Lead Researcher	Postdoc	Technician	Postdoc 2	Rent + Utilities	Reagents	Supply	Fume Hood	Apparatus	PM-NMR	iontorrent	DNA	sequencing	PK	XG	publications
1/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3/1/2014	366,375	366,375	4,375	0	0	0	3,000	3,000	1,000	100,000	40,000	200,000	0	15,000	0	0	0	0
4/1/2014	387,400	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
5/1/2014	408,425	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
6/1/2014	429,450	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
7/1/2014	450,475	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
8/1/2014	471,500	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
9/1/2014	492,525	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
10/1/2014	513,550	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
11/1/2014	534,575	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
12/1/2014	555,600	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
1/1/2015	576,625	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
2/1/2015	597,650	21,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
3/1/2015	728,675	131,025	4,375	4,700	4,700	0	3,000	3,000	1,000	0	10,000	0	100,000	50	200	0	0	0
4/1/2015	757,050	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
5/1/2015	785,425	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
6/1/2015	813,800	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
7/1/2015	842,175	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
8/1/2015	885,450	43,275	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	15,000	300	0	0	0
9/1/2015	913,825	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
10/1/2015	942,200	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
11/1/2015	970,575	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
12/1/2015	998,950	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
1/1/2016	1,027,325	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
2/1/2016	1,055,700	28,375	4,375	4,700	4,700	4,700	4,000	4,000	1,500	0	0	0	0	100	300	0	0	0
3/1/2016	1,160,175	104,475	4,375	4,700	4,700	4,700	4,000	0	0	0	0	0	0	0	0	36,000	36,000	10,000
Totals	1,160,175	1,160,175	109,375	112,800	112,800	56,400	87,000	83,000	29,500	100,000	50,000	200,000	100,000	31,600	5,700	36,000	36,000	10,000
Percent	93%	93%	9%	9%	9%	5%	7%	7%	2%	8%	4%	16%	8%	3%	0%	3%	3%	1%
category total			31%				7%	9%		36%				9%				1%

Employmer	31.31%
Physical Overhead	6.96%
Recurring Costs	9%
Research Infrastructu	36%
CRO/CRS	8.744%
Other	0.8%
margin	7.186%

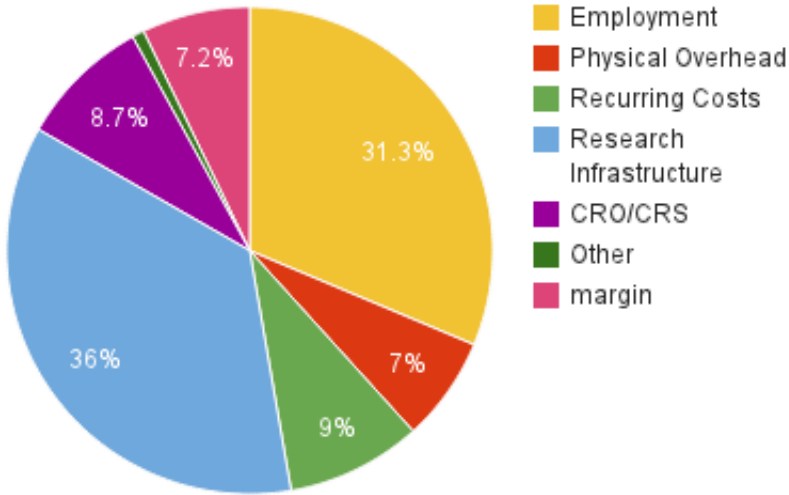
Funds:	1,750,000	Categories	Employment						Physical Overhead	Recurring Costs		Research Infrastructure				CRO/CRS				Other
Date	Running Total	Month Total	Lead Researcher	Postdoc	Technician	Postdoc 2	Technician 2	Postdoc 3	Rent + Utilities	Reagents	Supply	Fume Hood	Apparatus	PM-NMR	iontorrent	DNA	sequencing	PK	XG	publications
1/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2/1/2014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3/1/2014	366,375	366,375	4,375	0	0	0	0	0	3,000	3,000	1,000	100,000	40,000	200,000	0	15,000	0	0	0	0
4/1/2014	387,400	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
5/1/2014	408,425	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
6/1/2014	429,450	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
7/1/2014	450,475	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
8/1/2014	471,500	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
9/1/2014	492,525	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
10/1/2014	513,550	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
11/1/2014	534,575	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
12/1/2014	555,600	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
1/1/2015	576,625	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
2/1/2015	597,650	21,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	0	0	0	50	200	0	0	0
3/1/2015	738,675	141,025	4,375	4,700	4,700	0	0	0	3,000	3,000	1,000	0	20,000	0	100,000	50	200	0	0	0
4/1/2015	777,450	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
5/1/2015	816,225	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
6/1/2015	855,000	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
7/1/2015	893,775	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
8/1/2015	932,550	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
9/1/2015	986,225	53,675	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	15,000	300	0	0	0
10/1/2015	1,025,000	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
11/1/2015	1,063,775	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
12/1/2015	1,102,550	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
1/1/2016	1,141,325	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
2/1/2016	1,180,100	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
3/1/2016	1,218,875	38,775	4,375	4,700	4,700	4,700	4,700	4,700	5,000	4,000	1,500	0	0	0	0	100	300	0	0	0
4/1/2016	1,246,600	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
5/1/2016	1,274,325	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
6/1/2016	1,302,050	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
7/1/2016	1,329,775	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
8/1/2016	1,357,500	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
9/1/2016	1,385,225	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
10/1/2016	1,412,950	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
11/1/2016	1,440,675	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
12/1/2016	1,468,400	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
1/1/2017	1,496,125	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
2/1/2017	1,523,850	27,725	4,375	0	0	4,700	4,700	4,700	5,000	3,000	1,000	0	0	0	0	50	200	0	0	0
3/1/2017	1,681,325	157,475	4,375	0	0	4,700	4,700	4,700	5,000	0	0	0	0	0	0	0	0	60,000	60,000	14,000
Totals	1,681,325	1,681,325	161,875	112,800	112,800	112,800	112,800	112,800	159,000	120,000	42,000	100,000	60,000	200,000	100,000	32,250	8,200	60,000	60,000	14,000
Percent	96%	96%	9%	6%	6%	6%	6%	6%	9%	7%	2%	6%	3%	11%	6%	2%	0%	3%	3%	1%
category total			41%						9%	9%		26%				9%				1%

Employment	41%
Physical Overhead	9%
Recurring Costs	9%
Research Infrastructure	26%
CRO/CRS	9%
Other	1%
margin	4%

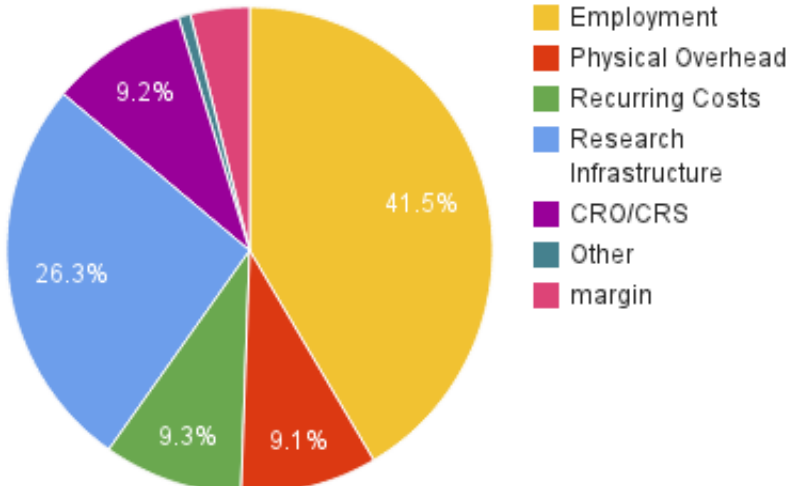
750k breakdown



1.25M breakdown



1.75M breakdown



Project Marilyn Timeline

January
Crowdfunding launch (January 7th)

February
February 7th: crowdfunding ends, unless extended up to two weeks depending on stretch goal status

Negotiate space requirements.

3rd week: indysci dot org Board Meeting (first board meeting after fundraising) Vote on making board meetings biannual moving forward, or dissolution of indysci if fundraising fails.

March 2014
Hires have moved to San Diego.
First meeting with outside advisor.
Begin paying hires.

July 2014
Complete modifications for Chemistry hood
*Complete evaluation of species (*M. smegmatis; S. avermitilis*) for synthetic bio work*
Complete evaluation of S. sibiricum *for continued work*

December 2014
Complete chemical evaluation for production of anthranilic acid analogs
*Complete evaluation of strains (*B. subtilis, S. cerevesiae*) for bioassay work*
Complete synthetic biology
Begin evolution on selected strains for yield improvement.

March 2015
Meeting with outside advisor. Go/no go; evaluation of likelihood of success in remaining timeframe.

July 2015
Negotiation with CROs for services
Begin scaleup of all compounds
Shipment of new compounds to NCI/60 cancer cell line panel

September 2015
Conclusion of Project Marilyn labwork. Shipment of compounds to CROs for evaluation.
(remainder of the time is general “flex-time” to account for experimental difficulties).

March 2016
Project Marilyn concluded.
Final meeting with outside adivsor. Evaluation of success condition or, go/no go to attempt to complete objectives with remaining funds.

Disbursal of remaining funds to selected 501(c)(3) hospital, and Project Marilyn stretch goals, if applicable.

Isaac T. Yonemoto

CONTACT INFORMATION

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EDUCATION

The Scripps Research Institute, San Diego, California USA

Ph.D., Chemistry, 2009

Thesis Topic: *Theory, methods, and investigations in amyloid dynamics*
Advisor: Professor William E. Balch
Co-advisor: Professor Jeffery W. Kelly
Area of Study: Biophysics and Chemical Biology

The University of Chicago, Chicago, USA

B.A., Mathematics, 2003

RESEARCH POSITIONS

Research Assistant November 2010 to present

Synthetic Biology and Bioenergy Labs, J. Craig Venter Institute

Reengineering *Alteromonas macleodii* hydrogenase for use in photosynthetic organisms. Development of techniques for prokaryotic genome manipulation.
Supervisor (PI): Professor Hamilton O. Smith
Co-PI: Professor Phillip Weyman

Research Associate September 2009 to August 2010

Department of Biochemistry, University of Maryland

Development and scaleup of a procedure to isolate a novel anticancer compound. Spectroscopic characterization and submission for biological assays.
Supervisor (PI): Professor Barbara Gerratana

Research Associate January 2004 to May 2009

Departments of Chemistry and Cell Biology, The Scripps Research Institute

Development of methods to synthesize amyloid peptides and studies on biophysical properties of amyloid peptides.
Supervisor (PI): Professor William E. Balch
Co-PI: Professor Jeffery W. Kelly

Research Associate June to December 2004

Department of Cell Biology, The Scripps Research Institute

Total Synthesis of Ubiquitin
Supervisor (PI): Professor Phillip E. Dawson

Research Assistant May 2001 to May 2004

Department of Chemistry, The University of Chicago

Synthesis of alkanethiol monolayer compounds
Supervisor (PI): Professor Milan Mrksirch

Refereed Journal Publications

Dual organism design cycle reveals small subunit substitutions that improve [NiFe] hydrogenase hydrogen evolution. **I. T. Yonemoto**, C. W. Matteri, T. A. Nguyen, H. O. Smith, P. D. Weyman. *J. Bio. Eng*, 2013, 7, 17

Mutasynthesis of a Potent Anticancer Sibiromycin Analogue. **I. T. Yonemoto**, W. Li, A. Khullar, N. Reixach, B. Gerratana. *ACS Chemical Biology*, 2012, 7, 6, 973

Cloning the *Acholeplasma laidlawii PG-8A* Genome in *Saccharomyces cerevisiae* as a Yeast Centromeric Plasmid. B. J. Karas, C. Tagwerker, **I. T. Yonemoto**, C. A. Hutchinson III, H. O. Smith. *ACS Synthetic Biology*, 2012, 1, 1, 22

The Importance of Single Molecular Determinants in the Fidelity of Expanded Genetic Codes. A. K. Antonczak, Z. Simova, **I. T. Yonemoto**, M. Bochtler, A. Piasecka, H. Czapinska, A. Brancale, E. M. Tippmann, *Proceedings of the National Academy of Sciences*, 2011, 108, (4), 1320

A General Strategy for the Bacterial Expression of Amyloidogenic Peptides Using BCL-XL-1/2 fusions. **I. T. Yonemoto**, M. R. Wood, W. E. Balch, J. W. Kelly. *Protein Science*, 2009, 18 (9), 1978

The 8 and 5 kDa Fragments of Plasma Gelsolin Form Amyloid Fibrils by a Nucleated Polymerization Mechanism, while the 68 kDa Fragment Is Not Amyloidogenic. J. P. Solomon, **I. T. Yonemoto**, A. N. Murray, J. L. Price, E. T. Powers, W. E. Balch, J. W. Kelly. *Biochemistry*, 2009, 48 (48), 11370.

Amylin Proprotein Processing Generates Progressively More Amyloidogenic Peptides that Initially Sample the Helical State. **I. T. Yonemoto**, G. J. A. Kroon, H. J. Dyson, W. E. Balch and J.W. Kelly. *Biochemistry*, 2008, 47 (37), 9900

Papers in Preparation

Interface site substitutions improve electron transfer from cyanobacterial ferredoxin to [NiFe] hydrogenase. **I. T. Yonemoto**, J. Jablanovic, P. D. Weyman.

Comprehensive substitution reveals patterns for Fe-S cluster ligation tolerance in [NiFe] hydrogenase. **I. T. Yonemoto**, B. R. Clarkson, P. D. Weyman.

Reviews

The Juggernauts of Biology. **I. T. Yonemoto**, E. M. Tippmann. *Bioessays*, 2010, 32 (4), pp 314-321

Other Scientific Activities

Founder, indysci.org

California registered nonprofit (C3489003) for the development of sibiromycin-based anticancer compounds, 501(c)3 status pending.