EXECUTIVE SUMMARY

GIVF/RIF Commercialization Program
The projects pair ISU faculty with Iowa companies to create or improve products or processes. Each project lasts two years. One year after the completion of the project (or three years after the start), the Iowa companies are surveyed for impact by the Center for Industrial Research and Service (CIRAS). These funds are a critical source of gap funding. They represent a unique resource that can be applied toward the success of Iowa companies. A summary of the projects funded to date is below, followed by the list of active projects. To date, 95 projects have been funded through the Commercialization Program. Eighty nine of these projects are complete and many show excellent progress in improving the competitiveness and profitability of the Iowa companies involved. Thirty startup companies have been assisted; including 18 new companies that were started in the first seven years as a direct result of the GIVF funding. In total more than 55 Iowa companies have participated in the program.

Surveys are conducted by CIRAS one year after project completion (true impact takes a minimum of 5-10 years).

<table>
<thead>
<tr>
<th>Project Dates</th>
<th>Survey Year</th>
<th>Companies Surveyed</th>
<th>Jobs Created or Retained</th>
<th>Total Sales Increase</th>
<th>Total Investment &amp; Cost Savings</th>
<th>Average Impact per Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY06-FY07</td>
<td>FY08</td>
<td>9*</td>
<td>71</td>
<td>$9,100,000</td>
<td>$23,500,000</td>
<td>$3,600,000</td>
</tr>
<tr>
<td>FY07-08</td>
<td>FY09</td>
<td>9</td>
<td>18</td>
<td>$3,700,000</td>
<td>2,760,000</td>
<td>720,000</td>
</tr>
<tr>
<td>FY08-09</td>
<td>FY10</td>
<td>8**</td>
<td>6</td>
<td>600,000</td>
<td>732,000</td>
<td>166,500</td>
</tr>
<tr>
<td>FY09 – FY10+</td>
<td>FY11</td>
<td>7**</td>
<td>13</td>
<td>675,000</td>
<td>967,000</td>
<td>234,571</td>
</tr>
<tr>
<td>FY10-FY11</td>
<td>FY12</td>
<td>6**</td>
<td>6</td>
<td>$1,750,000</td>
<td>$1,730,000</td>
<td>$580,000</td>
</tr>
<tr>
<td>FY11-FY12</td>
<td>FY13</td>
<td>2**</td>
<td>4</td>
<td>$260,000</td>
<td>$318,000†</td>
<td>$130,000</td>
</tr>
<tr>
<td>FY12-FY13</td>
<td>FY14</td>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All surveyed companies were start-up companies
** Surveys were not completed for all projects (not everyone chooses to participate in the survey)
† The sales increase was primarily from 1 successful project, but the jobs impact was spread. Many companies indicated it was too early to tell the sales impact (this is a frequent comment through the years).
†† Cost savings were not quantified by survey participants.

<table>
<thead>
<tr>
<th>Year Project Completed</th>
<th>Number of Projects</th>
<th>Number of Publications &amp; Presentations</th>
<th>Number of Invention Disclosures</th>
<th>Number of External Funding Applications</th>
<th>Number of Applications Awarded</th>
<th>External Funding Received*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY14+</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>$795,000</td>
</tr>
<tr>
<td>FY13</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>12</td>
<td>5</td>
<td>$6,364,000</td>
</tr>
<tr>
<td>FY12</td>
<td>11</td>
<td>50</td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>$940,000</td>
</tr>
<tr>
<td>FY11</td>
<td>11</td>
<td>46</td>
<td>3</td>
<td>20</td>
<td>6</td>
<td>$2,720,000</td>
</tr>
<tr>
<td>FY10</td>
<td>14</td>
<td>99</td>
<td>6</td>
<td>47</td>
<td>13</td>
<td>$3,500,000</td>
</tr>
<tr>
<td>FY09</td>
<td>15</td>
<td>53</td>
<td>4</td>
<td>48</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>FY07-08**</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

* Some information on award amounts was not included
** Data was not collected
* Partial results, projects are not complete
Proof of Concept Initiative
The GIVF/RIF funds have been incorporated into a Proof of Concept Initiative (POCI) http://www.industry.iastate.edu/POCI.html. The POCI is intended to build on the foundation started by the GIVF program, include additional funding sources such as i6, IPRT company assistance, Plant Sciences, etc., and position Iowa State to more rapidly propel technologies toward market opportunities. We will do this by emphasizing both the business opportunity and the technology in projects that are funded through the POCI. By doing this we will position young companies to be able to attract the next stage of funding from either the state, angel or VC sources and/or position technologies to be more attractive commercialization opportunities for existing companies.

There were an additional 16 projects funded under the POCI, using non-RIF funding sources. A grand-total of 111 projects have been funded through the POCI model from FY07 – FY13. Summary statistics for all POCI projects (RIF and all other funding sources) are as follows:

<table>
<thead>
<tr>
<th>Year Completed</th>
<th>Number of Projects†</th>
<th>Number of Publications &amp; Presentations</th>
<th>Number of Invention Disclosures</th>
<th>Number of External Funding Applications</th>
<th>Number of Applications Awarded</th>
<th>External Funding Received*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY14+</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FY13</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>6</td>
<td>$1,020,000</td>
</tr>
<tr>
<td>FY12</td>
<td>11</td>
<td>50</td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>$ 6,364,000</td>
</tr>
<tr>
<td>FY11</td>
<td>11</td>
<td>46</td>
<td>3</td>
<td>20</td>
<td>6</td>
<td>$   940,000</td>
</tr>
<tr>
<td>FY10</td>
<td>14</td>
<td>99</td>
<td>6</td>
<td>47</td>
<td>13</td>
<td>$ 2,720,000</td>
</tr>
<tr>
<td>FY09</td>
<td>15</td>
<td>53</td>
<td>4</td>
<td>48</td>
<td>20</td>
<td>$ 3,500,000</td>
</tr>
<tr>
<td>FY07-08**</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

†Includes all projects funded through the POCI
*Some information on award amounts was not included
+ Partial results, projects are not complete

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>FY12 GIVF Projects (completed May 31, 2013)</th>
<th>Award Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peter Keeling</td>
<td>Catalytic Conversion Platform for Furan Derivatives</td>
<td>$100,000</td>
</tr>
<tr>
<td>Tom McGee</td>
<td>Osteoceramic Bone Graft Pre-Clinical Evaluation for FDA Approval conducted at the University of Iowa based on Research and Patents at Iowa State University and licensed by ISURF to Osteoceramics, Inc. for Commercial Development</td>
<td>$ 92,074</td>
</tr>
<tr>
<td>Rick Sharp</td>
<td>Nutritional Intervention for Age-Related Muscular Function and Strength Losses</td>
<td>$99,44</td>
</tr>
<tr>
<td>Arun Asaithambi</td>
<td>Validation of Type 1 Diabetes Drug Candidate</td>
<td>$93,406</td>
</tr>
<tr>
<td>Zhiyou Wen</td>
<td>Development and Optimization of Pilot-scale Revolving Algal Biofilm Photobioreactor (RABP) for Easy Biomass Harvest</td>
<td>$50,000</td>
</tr>
<tr>
<td>Anumantha Kanhasamy</td>
<td>Small Molecule Non-receptor Tyrosine Kinase Inhibitors as Novel Neuroprotective Agents (part I)</td>
<td>$29,000</td>
</tr>
<tr>
<td>Eliot Winer</td>
<td>3D Visualization of Medical Data on Mobile Devices for Training, Diagnosis and Treatment (part I)</td>
<td>$35,000</td>
</tr>
</tbody>
</table>

FY13 RIF Projects (to finish May 31, 2014)
<table>
<thead>
<tr>
<th>Name</th>
<th>Project Description</th>
<th>Funding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eliot Winer</td>
<td>3D Visualization of Medical Data on Mobile Devices for Training, Diagnosis and Treatment (part II)</td>
<td>$15,000</td>
</tr>
<tr>
<td>Anumantha Kanthasamy</td>
<td>Small Molecule Non-receptor Tyrosine Kinase Inhibitors as Novel Neuroprotective Agents (part I)</td>
<td>$52,000</td>
</tr>
<tr>
<td>Zhiyou Wen</td>
<td>Development and Optimization of Pilot-scale Revolving Algal Biofilm Photobioreactor (RABP) for Easy Biomass Harvest—Phase II: Process Optimization and Algal Strain Evaluation</td>
<td>$50,000</td>
</tr>
<tr>
<td>Byron Brehm - Stecher</td>
<td>The MLVAnalyzer: Enabling a New Gold Standard</td>
<td>$50,000</td>
</tr>
<tr>
<td>Eve Wurtele</td>
<td>Bioassay-guided Fractionation to Isolate, Analyze and Characterize Therapeutic Compounds from <em>H. gentianoides</em></td>
<td>$50,000</td>
</tr>
<tr>
<td>Iver Anderson</td>
<td>Titanium Atomization Melt Delivery Tube Lifetime Assessment</td>
<td>50,000</td>
</tr>
</tbody>
</table>
Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report

**Report Type:** Final

**Title:** Catalytic Conversion Platform for Furan Derivatives

**PI:** Peter Keeling

**Company Partners (if applicable, company names only):** GlucanBio is now partnered with Nidus Partners who have become a co-owner.

**Project Goal:** The general goal is to evaluate technologies for converting monosaccharides and oligosaccharides to HMF leading to understanding the separation requirements for pre-pilot scale-up.

**Publications/presentations based on project:** None at this time.

**Invention disclosures:** None at this time, but ideas are emerging for an invention disclosure.

**External funding applied for (indicate received/denied/pending):**
- Received $200k funding from NSF as a PFI-AIR award (2011-13)
- Received $100k funding from Iowa GIVF award with matching funding from Nidus (2012-13)
- Received $150k funding from NSF as a SBIR-Phase I award (2013-2014)
- Pending $750k application for SBIR-Phase-II (2014-2015)
- Received and Pending ongoing funding from Nidus Partners and Founders (2012 and Future).

**Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):**

Glucan Biorenewables has continued to make great progress both technically and commercially and is looking well-placed to survive these early years and secure a path to consider commercial scale production in 2015/16.

This research project funded here focused on several purification strategies of (5-hydroxymethylfurfural) HMF from the organic phase of the biphasic reactor system. Care was taken to only research strategies that were potentially feasible at an industrial production scale. After reviewing pertinent literature and examining the chemical properties of the HMF versus the organic extraction phase, humins, and other contaminants, the decision was made to focus on the three means of purification; liquid-liquid extraction, adsorption onto a solid phase, and distillation.

The most productive means of purification attempted for HMF was by liquid-liquid extraction with water from the post reaction organic extraction phase. HMF has high solubility in pure water, but the salt used during the glucose to HMF dehydration reaction lowers the solubility of HMF in the aqueous phase. After the reaction is completed, the salt saturated aqueous phase is removed and replaced with fresh water allowing the liquid-liquid extraction to occur. The adsorption of HMF onto solid phase resins and the purification of HMF by distillation also both showed potential, but would require solvent changes detrimental to the glucose to HMF reaction yield. All three purification strategies could be viable options at production scale. Other factors in the production process of HMF will undoubtedly dictate the direction of HMF separation research.

On the commercialization side Glucan Biorenewables has secured a commercialization partner with Nidus Partners and is in the process of taking a license to the base technology at the core of this project. As a consequence of these commercialization steps, GlucanBio transformed itself into an LLC and Nidus became a member of the LLC with co-ownership rights. Nidus has done a great job on the business side of the company by developing commercialization models. GlucanBio recently secured an SBIR Phase-I grant from NSF. The company has also formed an additional partnership with a large corporation and is progressing towards pilot scale in 2014.
Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report

Report Type: Interim

Title: Small Molecule Non-receptor Tyrosine Kinase Inhibitors as Novel Neuroprotective Agents

PI: Anumantha Kanthasamy
Co-PI: George Kraus

Company Partners (if applicable, company names only): PK Biosciences

Project Goal: We propose to develop an orally active neuroprotective drug for the treatment of Parkinson’s disease in humans. The goals of this high impact exploratory study are to identify one or more novel RM108 derivatives that have lo-nanomolar potency, minimal off-target effects, metabolically stable and drug-like properties to initiate future advanced preclinical studies.

Publications/presentations based on project: None.

Invention disclosures: None.

External funding applied for (indicate received/denied/pending): An NIH R21 application will be resubmitted in Fall 2013.

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

We had proposed to design and synthesize one or more novel RM108 derivatives and validate them as small molecule Fyn kinase inhibitors. We designed and synthesized 10 structural analogs of RM108 that would possess physical characteristics for oral dosing and to enter the CNS. These RM108 analogs contain isosteric and other modifications that are important for Fyn kinase inhibition. These compounds expect to have drug-like properties with minimal structural liabilities. In vitro kinase screening by Invitrogen’s Z-lyte assay against 5 closely related kinases revealed that two new analogs selectively inhibited Fyn kinase by >70%. We also tested the neuroprotective efficacy of CL100 (15-45mg/kg, sub cutaneous, daily) in a sub chronic MPTP-treatment animal model of Parkinson’s disease. Results from this animal study revealed that RM analog CL100 protected against MPTP-induced motor deficits, striatal dopamine loss and nigral TH neuronal loss by more than 50%. For bioavailability studies, animals were injected with RM108 analog (15mg/kg, IV and SC) and then brain and plasma levels were measured at 1 hr. and 24 hr. by LC-MS/MS. Results from this study revealed that levels of RM108 analog was 5900-7000ng/ml in plasma and 33-78ng/g in the brain within 1hr of intravenous administration. Similarly, RM108 analog level was 3200-8300 ng/ml in plasma and detectable levels in the brain within 1 hr. of SC administration. These data suggest that the new RM108 analog selectively inhibited the therapeutic target Fyn kinase, was bioavailable in the brain, and was neuroprotective in animal models of PD. The data obtained in this study will help us build strong case of resubmission of our NIH R21 grant to further expand our CNS drug discovery project. We would like to thank Proof of Concept Initiative (POCI) of GIVF at Iowa State for their kind support.
Report Type: Final

Title: Identification and Characterization of Diabetes Drug Candidates for Type I Diabetes

PI: Arunkumar Asaithambi

Company Partners (if applicable, company names only): Signal Therapeutics

Project Goal: Identifying lead candidates for type 1 diabetes treatment

Publications/presentations based on project: None

Invention disclosures: ISURF#04048

External funding applied for (indicate received/denied/pending): 

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):
We have completed our studies to identify potential drug candidates for type 1 diabetes (T1D). Our data on the drug candidates in widely used pre-clinical T1D animal models show slowdown in the progression of type 1 diabetes (T1D). We see reduction in T1D features such as hyperglycemia, pancreatic beta cell death, weight loss, restoration of insulin production in streptozocin toxin model and non-obese diabetic (NOD) mice model. These studies have completed the specific aims listed in the GIVF proposal and has put our project towards lead optimization and pre-IND enabling studies stage. Signal Therapeutics is in the process of raising early-stage funding. We are in active discussions with several private investor and non-profit groups to advance the project towards clinical trials. Overall, we are making steady progress towards achieving our research and financial objectives.
Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report

Report Type: Interim

Title: Development and Optimization of a Pilot-Scale Revolving Algal Biofilm Photobioreactor

PI: Zhiyou Wen

Company Partners (if applicable, company names only): Gross Renewables

Project Goal:

To develop a novel attached algal culture system (Revolving Algal Biofilm Photobioreactor, RABP) for facilitating algal biomass harvest during algal biofuel production process.

Publications/presentations based on project:


Invention disclosures:

A Revolving Algal Biofilm Photobioreactor (RABP) for Easy Biomass Harvest, Submitted to ISU Research Foundation on 7/10/2012 (ISURF# 04050), Reassigned the right to the company on November, 2012. Filed by the company to US Patent and Trademark Office on 3/14/2013 (US patent application # 61/783,737)

External funding applied for (indicate received/denied/pending):

Development of a novel revolving algal biofilm photobioreactor (RABP) for easy biomass harvest. USDA-SBIR program, $100,000. Gross M (PI). 06/2013 - 12/2013. (Wen served as a PI of the ISU subcontractor of this SBIR proposal with a total budget of $33,333) (denied)

Development of a Novel Revolving Algal Biofilm Photobioreactor (RABP) for Easy Biomass Harvest. NSF-SBIR program. $150,000. Gross M (PI). 07/2013 - 12/2013. (Wen served as a PI of the ISU subcontractor of this SBIR proposal with a total budget of $50,000) (denied)

Production of Algae Biomass Using an Attached Growth System and Thermochemical Processing of Whole Algal Biomass into Fuel Intermediates.DOE-Algal Biomass Yield program. $3,685,360. 01/2014-06/2016/ (Wen served as PI of the project). (pending)

Bosch Carbon Nanofiber-algal Oil Polymer Composites. NASA-ESPCoR program. $750,000. 01/2014-12/2016. (Wen served as Co-PI of the project). (pending).

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

This project is focused on developing a novel biofilm based photobioreactor (Revolving Algal Biofilm Photobioreactor, RABP) which can be widely adapted by the algae industry for producing fuels and high value products. The RABP reactor can facilitate algal biomass harvest by reducing the harvest cost, which is a major
bottleneck in the commercialization of algal biofuel production. In the reporting period, we have performed a thorough lab-scale study to optimize the RABP operational conditions, so the algal biomass production yield can be reached to maximum. First, we evaluated a total 64 types of materials in terms of their capability of attaching algal cells, and found that duct cotton is the best materials because this material can attach the highest amount of algal cells on its surface and its excellent durability. Then, using the duct cotton as the attaching materials, we optimized the rotation speed of the RABP system, the algal biomass harvest frequency, and the CO₂ concentration used in the RABP system. Those optimization works lay the ground for developing a pilot scale RABP system for evaluating its commercial potential.

In the development of the pilot-scale RABP system, we constructed a green house in the BioCentury Research Farm in the reporting period, so the RABP system can be accommodated in the greenhouse for a year round operation. The greenhouse was a high premium facility with all the utilities and temperature control by a geothermal unit. Four RABP systems was then fabricated and assembled in the greenhouse. We have successfully run the RABP system for the algal culture in the green house for 6 months starting from January, 2014. Overall, the result shows that the pilot-scale RABP system produced a better result than the lab-scale RABP study due to the improvement of the light intensity. Also, the half-year data of pilot scale algal culture in the green house has shown the great improvement of algae biomass productivity achieved by the RABP system compared to the conventional open pond raceway system. In the remaining project period, we will continue running the RABP system for getting year around algae growth performance so a thorough evaluation of the RABP system can be obtained.
Report Type: Final

Title: Nutritional Intervention for Age-Related Muscular Function and Strength Losses

PI: Rick Sharp

Company Partners (if applicable, company names only): MTI

Project Goal: Examine the effectiveness of vitamin D plus hydroxyl-methylbutyrate dietary supplementation in promoting muscle strength and functionality improvements in older adults during 12 weeks of a strength training program.

Publications/presentations based on project: none

Invention disclosures: none

External funding applied for (indicate received/denied/pending):

NIH – SBIR Phase I grant in collaboration with Metabolic Technologies, Inc. Nutritional Intervention for Age-Related Muscular Function and Strength Losses. Funded, $50,000.

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

Our original proposal was to recruit and test 50 research participants (25 men, 25 women) above 60 yrs. of age from the central Iowa area. At present, we have completed 46 individuals and the final four participants will complete the 12 week intervention by August 1, 2013. Because this research involves dietary supplementation, the interventions must be conducted double blind. Consequently, we must wait until all participants have completed the protocol before evaluating effectiveness. Once the last of the current participants has finished the intervention, we will analyze the results for significant differences and transmit a data report to the NIH data safety monitoring board that oversees this project. Based on our analysis and the assessments of data safety monitoring board, we will begin Phase II of the project which has received preliminary approval from NIH. We anticipate starting the Phase II trials by January 2014.
Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report

Report Type: Final

Title: Osteoceramic Bone Graft Pre-Clinical Evaluation for FDA Approval

PI: Tom McGee/Kris Johansen

Company Partners (if applicable, company names only): University of Iowa

Project Goal:
Determine the effect of OsteoCeramics ceramic implant (OC-Ceramic) on bone regeneration in a rabbit tibial defect model through the use of plain radiography, pqCT, histology, and mechanical testing.

Publications/presentations based on project: Effects of osteoceramics on bone regeneration; Partnering for Growth, March 27, 2013, Ankeny, IA

Invention disclosures: None

External funding applied for (indicate received/denied/pending):

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

OC-Ceramic has potential for use as artificial off-the-shelf bone grafts to replace currently used materials and has advantages of being able to help guide bone growth and bone promote attachment to the implant to prevent mechanical failure seen with current implants. FDA approval will be required before the OC-Ceramic material can be used in humans. This GIVF project is directed at pre-clinical evaluation on rabbits performed at the Bone Healing Research Lab-Iowa Spine Research Center (BHRL/ISRC), Department of Orthopaedics and Rehabilitation, University of Iowa Carver College of Medicine. The evaluation includes two time points (6 and 8 weeks) in a rabbit tibial defect model. Results from the 6 and 8 week time points indicate that the OC-Ceramic material has better strength than control material (natural bone graft taken from the patient). The results are being tabulated in preparation for submitting a proposal to the FDA for 510(K) approval.
Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report

Report Type: Interim

Title: Visualization of Medical Data on Mobile Devices for Training, Diagnosis and Treatment

PI: Eliot Winer

Company Partners (if applicable, company names only): Visual Medical Solutions, LLC

Project Goal: To research and commercialize volume rendering of medical data on a mobile device


Invention disclosures: ISURF Disclosure #4004 – licensed to Visual Medical Solutions, LLC.

External funding applied for (indicate received/denied/pending): None

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

A prototype iOS application has been developed during the time of the project. This allows a user to load in a computed tomography (CT) or Magnetic Resonance Imaging (MRI) dataset and view it in interactive 3D. A user can fully manipulate the representation through rotation, translation, scaling, coloring, and displaying of different tissue densities (e.g., bone, fat-bone range, etc.). This was accomplished by making novel advancements in techniques for orthogonal texture slicing and memory bandwidth optimization. These were then applied to an iOS device to create the prototype. This entailed tuning the graphics processing unit (GPU) operations so that interaction remained real-time for a user.

In addition, work was done on the user interface so that loading and accessing data would be as simply and intuitive as possible. The labeling of different modes (i.e., tissue types, coloring, etc.) and artwork were created by the company partner on the project. Bug testing by users revealed some inherent preferences for the user interface, some of which are currently being implemented. The company partner has begun working with the research code to “harden” it up for release which is planned, at this point, for late 2013 or early 2014.
RIF FUNDING: PROGRESS REPORT

Report Type: Interim

Title: MLVAnalyzer™: Enabling a New “Gold Standard” for Bacterial Strain Typing

PI: Dr. Byron Brehm-Stecher

Company Partners (if applicable, company names only): Advanced Analytical Technologies, Inc. (AATI)

Project Goal: To validate the performance and capabilities of AATI’s newly developed MLVAnalyzer™ parallel capillary electrophoresis system. Elements to be examined include this instrument’s reproducibility, discriminatory power, ease of use and comparability of results to existing high-cost MLVA analysis systems. This validation will be performed in Dr. Brehm-Stecher’s Rapid Microbial Detection and Control laboratory using Salmonella as a model organism.

Publications/presentations based on project:
Poster presentation: American Society for Microbiology General Meeting, May 18-21, 2013, Denver, Colorado
Title: “Use of a Low-Cost Multi-Color Fluorescence Capillary Electrophoresis Unit for the Differentiation of Salmonella species”

Invention disclosures: none this period.

External funding applied for (indicate received/denied/pending): Immediately prior to application to RIF, funding was sought from USDA and NSF Phase I SBIR programs. Neither grant was awarded, but some reviewer comments were encouraging. We plan to resubmit to the USDA program closing September 26th, 2013. We may utilize the Iowa Innovation Corporation’s pre-proposal screening service as a means to obtain additional pre-submission feedback prior to this submission.

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

The MLVAnalyzer™ (Advanced Analytical Technologies, Inc, Ames, Iowa) allows for rapid strain tracking of pathogens using traditional multiplex PCR amplification and identification of Short Tandem Repeats (STRs) using a multicolor fluorescence capillary electrophoresis system. The CE system is competitively priced with pulsed-field electrophoresis systems, and allows smaller laboratories to apply the powerful MLVA technique for pathogen identification. We examined the feasibility of this technology for routine strain identification and differentiation of Salmonella isolates.

DNA was extracted from 10 ATCC cultures and 25 isolates of Salmonella Typhimurium identified through traditional testing. End-labeled fluorescent primers against 5 VNTR (Variable Number Tandem Repeat, a type of STR) regions on the Salmonella genome were synthesized. Multiplex PCR amplifications were performed (2 reactions per isolate), generating 5 differently-sized PCR products. These end-labeled fragments were separated by this multiplexed capillary gel electrophoresis system with fluorescence detection and a user-compiled pattern library was created.

Multiplexed PCR targeted to the STR regions in the Salmonella genome produced amplified products ranging in size from approximately 150 - 500 base pairs. These products were adjusted to a working dilution and loaded onto 96-well PCR plates for analysis. The plates were analyzed using the newly developed The MLVAnalyzer™ capillary electrophoresis instrument. Products migrated based on size and were detected by fluorescence from incorporated primers. The software provides rapid data processing and interpretation, enabling accurate, automated differentiation of MLVA patterns from the S. Typhimurium strains analyzed.
The MLVAnalyzer™ developed and validated during this rapid concept-to-solution granting period provides reliable STR-based strain identification for outbreak investigations, source identifications and dendrogram mapping studies. Our work has validated the hardware needed to unlock MVLA’s potential for broader use in industry for typing of this and other bacterial pathogens. This represents the first step toward implementing a rapid, equivalent cost replacement for Pulsed-Field Gel Electrophoresis (PFGE), the current “gold standard” typing approach. We expect that availability of this resource will ultimately result in broad usage/acceptance among various users, including The Centers for Disease Control and Prevention (CDC), state public health laboratories, International PulseNet participants, additional Federal agencies with mandates for tracking of bacterial pathogens, world health organizations and pharmaceutical companies. Ultimately, we expect this enabling technology will ease pathogen tracking, promote timely intervention of disease and will serve as an important tool in the ongoing effort to reduce the human, economic and sociological burdens of bacterial disease.
**RIF FUNDING: PROGRESS REPORT**

<table>
<thead>
<tr>
<th>Report Type:</th>
<th>Interim</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title:</strong></td>
<td>Bioassay-guided Fractionation to Isolate, Analyze and Characterize Therapeutic Compounds from <em>H. gentianoides</em></td>
</tr>
<tr>
<td><strong>PI:</strong></td>
<td>Eve Wurtele</td>
</tr>
<tr>
<td><strong>Company Partners (if applicable, company names only):</strong></td>
<td>BioScience Research Capital, I.I.C.</td>
</tr>
<tr>
<td><strong>Project Goal:</strong></td>
<td>The development of therapeutically beneficial nutraceutical extracts and compounds from <em>Hypericum gentianoides</em>.</td>
</tr>
<tr>
<td><strong>Publications/presentations based on project:</strong></td>
<td>none yet</td>
</tr>
<tr>
<td><strong>Invention disclosures:</strong></td>
<td>none yet</td>
</tr>
<tr>
<td><strong>External funding applied for (indicate received/denied/pending):</strong></td>
<td>none yet</td>
</tr>
</tbody>
</table>

**Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):**
The first portion of the project has involved the collection of seeds and growth of plant material. In addition we have initiated studies on the appropriate methods for purifying the compounds needed. During this time, we have ruled out the use of the preparative column that we initially had hoped to use, and are now working with a semi-preparative column that will give us greater resolution. In addition, we have identified an independent company which can independently analyze the samples that we provide them, and have established the general protocol for analysis.
RIF FUNDING: PROGRESS REPORT

Report Type: Interim

Title: Titanium Atomization Melt Delivery Tube Lifetime Assessment

PI: Iver Anderson

Company Partners (if applicable, company names only): Iowa Powder Atomization Technologies, Inc. (IPAT)

Project Goal: The primary goal of this project was to test the ability to integrate IPAT’s licensed pour tube into a commercial capacity titanium melting system with a different heating configuration compared to that established at Ames Laboratory. If successful integration is realized, testing of the tube lifetime will be conducted by pouring molten titanium through the tube and analyzing the resulting metal and pour tubes.

External funding applied for (indicate received/denied/pending): Previous work on a prototype close-coupled titanium gas atomizer has been generated by multiple funding sources to allow for the work being conducted within the scope of this project.

- Subcontract entitled: “Feasibility Tests for Large Scale Advanced Titanium Powder Production,” for $830,000 as part of the full proposal, “Near Net Shape Manufacturing For Current and Future Generation Munitions and Armament Systems ($270,000, program only active 1 year).
- Proposal entitled: “Design and Completion of Advanced Titanium Gas Atomizer,” through the Iowa State University Research Foundation for $25,000 (received).
- Proposal entitled: “Supplemental Support of Advanced Capability for Titanium Melting,” through the Iowa State University Research Foundation for $25,000 (received).
- Supplement to subcontract award entitled: “Development of Gas Atomization System to Produce Fine Spherical Titanium Powder,” under Northern Illinois University for $20,000 (received).

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):
A series of IPAT licensed pour tubes with different geometries were fabricated at Ames Laboratory for use in a commercial-size titanium melting system. Discussions with the third party melting company allowed for the correct geometrical changes for integration into the third party melting system. The pour tubes were brought by Ames Lab personnel on-site to the third party for a week-long series of experiments. A sub-set of the pour tubes were placed into the on-site commercial melting system; no titanium metal was present in the melting crucible. This was done to allow for observation of the tube with the commercial heating configuration. Of the sub-set of tubes tested in this manner, the best performing geometry was chosen for further melting and casting experiments.

A casting trial was conducted using Ti-6Al-4V with a pour tube of previously-chosen geometry. After the charge was fully molten, the metal began to pour in approximately one minute. The pour lasted for 4 minutes and 38 seconds before the melt stream veered to one side of the casting mold and the run was stopped. The ingot from the casting trial weighed 40.5 pounds. A central slice was taken from the middle of the resulting ingot. The solidified titanium and the pour tube used for the casting were also collected. The poured Ti-6Al-4V and the pour tube from the first casting trial were brought back to Ames Lab for further analysis. Three pieces from the central slice were sent out for wet chemistry analysis along with unmelted material. Wet chemistry by independent analysis provided evidence that the pour tube liner can maintain chemical purity for the duration of the initial titanium casting.
<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>FY12 i6 Projects (completed May 31, 2013)</th>
<th>Award Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martin Spalding</td>
<td>Enhancing Photosynthetic CO₂ Assimilation and Biomass Accumulation in Vascular Plants by Transgenic Expression of Microalgal CO₂-Concentrating Mechanism Genes (Phase I and Phase II)</td>
<td>$99,893</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>FY13 i6 Projects (to finish May 31, 2014)</th>
<th>Award Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>George Kraus</td>
<td>Bio-based Production of Terephthalic Acid and other Aromatic Molecules (Phase I and Phase II)</td>
<td>$100,000</td>
</tr>
<tr>
<td>Basil Nikolau</td>
<td>SoLysTE: A start-up focused on novel biocatalysts for the production platforms of diverse fatty acid products (Phase I)</td>
<td>$50,000</td>
</tr>
<tr>
<td>Basil Nikolau</td>
<td>Characterization of Biocatalysts for Novel Production Platforms for Diverse Bi-functional Precursors of Polymers and Surfactants (Phase I)</td>
<td>$50,000</td>
</tr>
<tr>
<td>Alex Stoychev</td>
<td>Reducing the Total Energy Footprint of Popular Mobile Apps Through Better Algorithms (Phase I)</td>
<td>$50,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>FY13 PSI Projects (to finish May 31, 2014)</th>
<th>Award Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas Lubberstedt</td>
<td>Development of Midwest-adapted and specialty inducer for haploid production in corn (Phase I and Phase II)</td>
<td>$55,235</td>
</tr>
</tbody>
</table>
Report Type: Interim

Title: Bio-based Production of Terephthalic Acid and other Aromatic Molecules

PI: George Kraus

Company Partners (if applicable, company names only): SusTerea

Project Goal: Ultimately the aim is to make coumalic acid into a platform molecule with a range of chemical outcomes.

Publications/presentations based on project:

Invention disclosures:
ISURF 04029

External funding applied for (indicate received/denied/pending):
SECO, 2102, denied
AIR 2012, denied
SECO, 2013, pending

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

Terephthalic acid is a commodity chemical produced from petroleum feedstocks. The most common synthesis pathway is the oxidation of \( \text{para-xylene} \) using transition metal catalysts. Terephthalic acid and dimethyl terephthalate are employed in the preparation of polyethylene terephthalate (PET), a thermoplastic polymer used in many beverage and food containers and in fabrics, and polytrimethylene terephthalate, a material used in carpets and upholstery. Global production of terephthalic acid was over fifty million tons in 2009. An effective, green route to terephthalic acid could have a large impact. Coumalic acid is a key intermediate in our approach to terephthalates and benzoic acid-based surfactants. We recently reported the regioselective Diels-Alder reactions of coumalic acid with alpha-olefins. The process involves a Diels-Alder reaction to produce a bicyclic intermediate that can be dehydrogenated in situ by a catalyst to form the \( \text{para} \)-substituted benzoic acid in 99\% \( \text{para} \)-selectivity. In order for this remarkable transformation to become industrially useful, a viable and scalable synthesis of coumalic acid is needed.

Since a strong acid and heat will be needed to protonate malic acid, we examined several strong anhydrous acids. Using acetic acid or trifluoroacetic acid without sulfuric acid present gave small amounts of O-acylated products and returned starting material. The more strongly acidic sulfonic acids, triflic acid and nonafluorobutanesulfonic acid, gave coumalic acid in good yields, while methanesulfonic acid gave mixtures of 1 and 3. With the best conditions discovered to date, we scaled up the reaction with triflic acid and obtained an 86\% yield of coumalic acid on a five-gram scale.
i6 FUNDING: PROGRESS REPORT

Report Type: Interim

Title: SoLysTE: A startup focused on novel biocatalysts for the production platforms of diverse fatty acid products

PI: Basil Nikolau

Company Partners (if applicable, company names only):

Project Goal: This i6-Green project will leverage our previously established screening platform to identify thioesterase enzymes specific for producing fatty acids at each specific chain length. Each of these fatty acids has potential commercial interests with different industrial and food applications, represented by several CBiRC company members. Moreover, the project will build the fundamental basis for a start-up company based on these technologies, now named VariFAS Biorenewables.

Publications/presentations based on project:

Invention disclosures:

External funding applied for (indicate received/denied/pending): Results from this project will be included in a grant application to the NSF-SBIR program due Fall, 2013.

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

In the current funding period, our goal was to determine functionality of 25 thioesterases (isolated from diverse biological sources) in terms of enzymatic activity and fatty acid productivity using our established screening protocols (Jing et al., 2011). We selected genes encoding for 30 uncharacterized bacterial thioesterases, and these were commercially synthesized. A total of 27 thioesterases were successfully evaluated for the types of fatty acids that they can generate in our established screening platform. This collection of thioesterases provided novel fatty acid profiles, including some that showed distinct preferences for producing short chain fatty acids (<8 carbon atoms) and others that produced equal amounts of fatty acids and fatty acid derivatives (methylketones). Other thioesterases produced appreciable quantities of unsaturated fatty acids, which have potential as feedstocks for straightforward chemical conversion to alpha-olefins. For example, the primary products for one thioesterase were butanoic and butenoic acids, comprising ~65% of total fatty acid production. Biological production of butenoic acid is of potential commercial interest, because it can be chemically converted to the alpha-olefin, propylene, which is currently produced from petroleum-based feedstocks and is widely used by industry to produce polypropylene and other downstream chemicals. Butanoic acid has potential commercialization application as an antimicrobial agent in food preservation.
**Appendix 1: FY13 Board of Regents Annual Economic Development and Technology Transfer Report**

### i6 FUNDING: PROGRESS REPORT

**Report Type:** Interim

**Title:** Characterization of Biocatalysts for Novel Production Platforms for Diverse Bi-functional Precursors of Polymers and Surfactants

**PI:** Basil Nikolau

**Company Partners (if applicable, company names only):** OmegaChea Biorenewables LLC

**Project Goal:**

Purification and characterization of ten diverse KASIII genes to identify enzymes with maximum activities with specific substrates.

**Publications/presentations based on project:**

- Poster presentation at the 2013 ASBMB Meeting, April 2013, Boston, MA
- Poster presentation at the 5th Annual CBiRC NSF Site visit meeting, May 2013, Ames, IA

**Invention disclosures:** ISURF 04083

**External funding applied for (indicate received/denied/pending):**

- NSF STTR Phase I Award (Received – July 2013)

**Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):**

In this project, we successfully expressed and purified 3-Ketoacyl ACP Synthase III enzymes from ten diverse biological sources. Our aim was to identify specific KASIII enzymes that have maximum activities with different starter substrates, and thereby develop the catalytic technology to produce different fatty acid products. We conducted *in-vitro* enzyme assays on each of the ten diverse KASIII enzymes to ascertain the activity and substrate specificity of these enzymes with various acyl-CoA starter substrates, including straight chain, branched chain and hydroxylated acyl CoAs. Our data showed that five KASIIIs exhibited comparable activities with branched chain substrates. These KASIIIs could be used in an engineered system to produce branched chain fatty acids, which have utility as surfactants and lubricants at low temperatures. In addition, two KASIIIs exhibited the highest activities on hydroxylated substrates. Such KASIIIs can be used for production of novel hydroxylated fatty acids, which have potential applications in polymers, surfactants and lubricants.

To commercialize KASIII technology, we conducted market feasibility analysis and developed a business model and a business plan, and also sought funding from various state and federal sources. OmegaChea won the Pappajohn Student Business Plan Competition in May 2013 and is currently competing in the Pappajohn Iowa Business Plan Competition. OmegaChea also secured the NSF STTR Phase-I award that will help develop a platform for production of OmegaChea’s first bi-functional fatty acid product. Upon completion of the Phase I award, OmegaChea envisions applying for a STTR Phase II award, and also partnering with another firm to take the technology closer to the market place.
Report Type: Interim

Title: Reducing the Total Energy Footprint of Popular Mobile Apps Through Better Algorithms

PI: Alex Stoytchev

Company Partners (if applicable, company names only): N/A

Project Goal: Develop a proof-of-concept application that shows the feasibility of our approach for reducing the energy footprint of popular mobile apps.

Publications/presentations based on project: In preparation.

Invention disclosures: No new disclosures.

External funding applied for (indicate received/denied/pending): None yet.

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

The goal of this project is to investigate if a new class of algorithms can be used to reduce the total energy footprint of some popular smart phone applications. As promised, we developed a proof-of-concept application that runs in real-time on an Android phone. The application is stand alone, i.e., it runs only on the smart phone and does not require external resources, which consume power at a remote location. For example, it does not require a cellular network or even an Internet connection in order to function properly. Off-line tests to improve the speed of the new class of algorithms even further were also performed and were successful. The proof-of-concept prototype is ready and it should help with the commercialization prospects of this technology.
Title: Enhancing Photosynthetic CO₂ Assimilation and Biomass Accumulation in Vascular Plants by Transgenic Expression of Microalgal CO₂-Concentrating Mechanism Genes

PI: Martin H. Spalding

Company Partners (if applicable, company names only): N/A

Project Goal: Improve photosynthesis and productivity in crop plants by enhancing CO₂ supply rate to the limiting photosynthetic enzyme, Rubisco, using elements of the microalgal CO₂-concentrating mechanism.

Publications/presentations based on project: None yet

Invention disclosures: None yet

External funding applied for (indicate received/denied/pending): None yet

Progress report (300 word maximum, please focus on results in non-technical terms and commercialization progress):

Our overall goal is to offset the deficiencies of CO₂ assimilation in important crop plants by incorporating components of an algal CO₂-concentrating mechanism (CCM) into Arabidopsis and rice. We postulated that expression of 3 Chlamydomonas CCM genes (LCIA, LCIB and LCIC) in crop plants might enhance their photosynthesis and yield. This project was proposed in two, 6-month stages; the first 6-month milestones were met. The 12 month milestones are:

1. Demonstrate protein-level expression and cellular localization of transgenes in T1 or T2 generation progeny of rice, both individually and in combinations.
2. Evaluate growth and carbon fixation rates in Arabidopsis progeny (T3) and rice progeny (T2 or T3) homozygous for expression of transgenes, individually and in combination.
3. Provide preliminary Proof-of-Concept that partial expression of a microalgal CCM mechanism can deliver increased carbon fixation in plants.

We also included a 4th CCM gene, HLA3 in the project. Protein level expression and proper localization was demonstrated for genes LCIA, LCIB, LCIC and HLA3 in T1 and T2 generations of the model plant Arabidopsis and in rice. LCIA expression in both Arabidopsis and rice resulted in a stunted phenotype with no obvious photosynthetic differences. Although clearly not the effect we were seeking, this result does demonstrate that the LCIA protein is functional in plants and will facilitate basic research on its function. No obvious phenotypes were observed for homozygous transgenic Arabidopsis or rice expressing LCIB, LCIC, LCIB+LCIC, or HLA3. Preliminary data indicate an increase in seed weight for the rice HLA3, LCIB and LCIB+LCIC transgenic lines, although the data were from bulked seeds. More detailed growth chamber experiments are ongoing to try to determine whether the increased seed weight is replicable and affects seed yield per plant.
PSI FUNDING: PROGRESS REPORT

Report Type: Interim

Title: Development of Midwest-adapted and specialty inducer for haploid production in corn

PI: Ursula Frei; Thomas Lubberstedt

Company Partners (if applicable, company names only):

Project Goal: Developing haploid inducing genotypes adapted to different environments and applicable in specialty corn

Publications/presentations based on project:

Invention disclosures:
ISURF #4065 – Lubberstedt, Thomas – Development of a haploid inducing genotype (inducer) adapted to the Midwest for maize
ISURF#04099 – Lubberstedt, Thomas - Haploid inducing genotype for specialty corn

External funding applied for (indicate received/denied/pending):

Progress report:

Mid West Adapted Inducer (MWID):
10 promising lines (F5/F6) related to B73 were tested during winter season 12/13 for their induction ability, using a commercial hybrid as tester. For three lines the induction rates were as high as for the RWS/RWK-76 inducer (reference genotype), based on screening a minimum of 3000 induced kernels per line. The bulked offspring of these three promising lines will be tested in summer 2013 again for their induction rate in a commercial hybrid and inbred lines representing the different heterotic groups in maize. Agronomic traits will be recorded and as much seed as possible produced for the planned release. Final induction data will be available towards the end of fall 2013. A release is planned for end of 2013.
F3 and F4 families of additional inducer lines in other genetic backgrounds than B73 will be tested this summer for their induction ability for the first time.

Specialty Inducer:

a) Popcorn Inducer (PCI)
From the 22 single ear descents that were tested in winter 12/13 for their induction ability on popcorn maize as tester, six showed promising induction rates. Single ear descents from genotypes with favorable tassel traits were selected within these families. 32 resulting families will be tested this summer for their induction rates using a commercial hybrid and a popcorn as testers. The focus is on progenies that have an additional root color marker for haploid selection, as selection based on R1-nj alone seems to be difficult in popcorn. The materials planted this summer are F2 populations. Thus, at least another two generations of self-pollination and selection will be necessary to obtain a reasonably stable line for release (fall of 2014).

b) Indian Corn Inducer
Indian Corn is used for the production of natural colors. The conventionally used marker genes for haploid selection cannot be used in this heavily colored genetic background. During winter 12/13 we started test crosses between Indian Corn and several genotypes bearing novel selectable marker genes, which we intend to introduce into our haploid inducer program. These experiments will be continued during summer 2013.