



Scientific Update Summary of TGen's Ewing's Sarcoma Research- September 2008

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Ewing's sarcoma is a form of bone cancer which affects young adults, most commonly occurring in the pelvis, femur, humerus, and ribs. Surgery to achieve local control of the disease, combined with aggressive chemotherapy has dramatically improved the clinical outcome for patients, but current therapy is imperfect. There is a great unmet need for more effective therapeutics to better control local disease, and particularly important for improving the survival of patients with metastatic disease.

The goal of TGen's Ewing's sarcoma research project is to apply state of the art molecular and genomic technologies to discover new gene targets, and advance new biological drugs to achieve more complete control of local disease. The research project at TGen is composed of two parallel efforts:

The first effort is focused on finding new gene targets that could be used for drug discovery. Specifically, our team is applying unique high throughput RNAi technologies to knock down genes across the genome of Ewing's sarcoma cells to discover genes that these cancer cells are dependent on for survival. Although the data from these screens is still being generated and analyzed, preliminary results have already led us to identify several gene knock outs which effectively kill Ewing's sarcoma cells, representing interesting points of vulnerability that can be used for drug targeting. A noteworthy and validated 'hit' in the functional screen is the IGF-1R gene target. Interestingly, other research efforts have also implicated this gene in Ewing's sarcoma biology, further strengthening the evidence for the use of emerging inhibitors to IGF-1R in Ewing's sarcoma therapy. Ongoing mining of the data from the screening component of this project are expected to implicate additional targets and insights into vulnerabilities that could be therapeutically exploited.

The second effort is focused on exploiting a known genetic alteration, which is specific to Ewing's sarcomas. This alteration produces a fusion between two normal proteins resulting in a new aberrant fused protein that drives the growth and survival of Ewing's cancer cells. The specific goal of this second aspect of the project is to leverage the capabilities and technologies developed in Dr. David Azorsa's biological therapeutics laboratory to create highly specific monoclonal antibodies which can bind and inhibit the Ewing's fusion protein. These antibodies represent potential drugs with 'magic bullet' like specificity for cancer cells expressing the fusion protein. The expectation is that these antibodies will eventually be useful as biological drugs to block the growth and survival of Ewing's sarcoma cells but sparing all other normal cells in the body. Currently, the necessary reagents have been developed, immunization experiments have already produced antibody-producing cells (hybridomas), and antibodies are currently being screened to identify and prioritize antibodies with the appropriate functional activity to qualify as drug candidates that could be advanced towards pre-clinical and clinical development.

The two aspects of the project are currently progressing on time according to schedule, with very promising preliminary results that indicate that: 1) the project will successfully achieve the expected new insights into vulnerable gene targets, and 2) the project is progressing as expected towards generating a new biological drug with selectivity for Ewing's sarcoma cells.

In conclusion, the generous research funding given for this project has effectively and efficiently been leveraged to support important basic research that is on track towards producing a significant impact in the battle against Ewing's sarcoma. Thank you for joining us towards making a meaningful difference.

Additional noteworthy events and milestones:

Establishment of Ewing's sarcoma cell line models and reagents to be used in both parts of the project:

Two Ewing's Sarcoma Type I cell lines were obtained from an NCI-NIH collaborator (Dr. Javen Khan)

Two Ewing's Sarcoma Type II cell lines were obtained from the ATCC

Immunizing chimeric peptides were designed to target the Type I fusion protein sequence

Preliminary Screening Results:

A high throughput knock out assay was developed

The first round of data led to the discovery of IGF-1R and several other candidate gene targets that are vulnerable in a E.S. Type I cell line.

Additional screening and further validation of new targets is underway.

Biological Therapeutic Antibody Development

Immunizations were carried out to produce anti-EWS-FLI1 monoclonal antibody producing cells
Current efforts are now focused on screening hybridomas to discover and validate specific antibodies which have high affinity binding and are functionally active.

April 18th, 2008: Ewing's Research Foundation visits TGen's Scottsdale Mayo Clinic Campus facility to meet with the Ewing's research team to review project progress. TGen Scientists in attendance included:

David Azorsa, Ph.D, Senior Investigator PGD, TGen, Head of Ewing's Project

Jeff Trent, Ph.D., President & Scientific Director, TGen

Daniel Von Hoff, M.D, Executive VP and Director Translational Drug Development Division (TD2)

Michael Barrett, Ph.D, Investigator, Head of Oncogenomics Lab

Spyro Mousse, Ph.D, Director, Pharmaceutical Genomics Division (PGD)

September 25th to 27th, 2008: Dr. Spyro Mousse presented a seminar to share preliminary unpublished data and results from TGen's Ewing's research project to the sarcoma research community at the 2008 AAOS/ORS Molecular Biology & Therapeutics in Musculoskeletal Oncology Research Symposium, and international scientific conference, in Salt Lake City, UT.