The Role of the Gut Microbiome on the Anti-Tumor Response of Immune Checkpoint Inhibitors in a Syngeneic Murine Model of HPV-Associated Cancer
TD2 – The Precision Oncology CRO™

- Located in Scottsdale, AZ
- Support for preclinical through Phase II oncology clinical trials including regulatory affairs
- Collective experience in performing Phase I studies on >400 new anti-cancer agents
- AAALAC Accredited Facility
- In Vitro/In Vivo Pharmacology and DMPK Studies

Mayo Clinic Collaborative Research Building
(Located in Scottsdale, AZ on the campus of the Mayo Clinic Arizona)
Study Goals

► Validate a syngeneic model of HPV-associated cancer and characterize the activity of immune checkpoint inhibitors

► Confirm and extend published data on Bifidobacteria supplementation on the enhancement of anti-cancer activity of immune checkpoint blockade

► Determine the combination benefit of a small molecule epigenetic immunomodulator combined with Bifidobacteria supplementation
Enhancement of anti-αPD-L1 activity by Bifidobacterium addition to treatment regimen

Data Presented by Sivan, et.al Science 2015

Study Conducted at TD2 in 2015
Characterization of the TD2 Mouse Microbiome

- The mouse microbiome varies between animal facilities contributing to variability in syngeneic tumor growth rates and immune checkpoint inhibitor performance across institutions
  - We have characterized the TD2 microbiome using 16S ribosomal RNA (rRNA) gene sequencing
  - We have defined the time required for study animals to adopt the TD2 microbiome
  - Stable and known microbiome could reduce study variability and inhibitor activity

**Taxonomy: Order – Change Over Time Assessment**
Syngeneic Model of HPV-Associated Cancer

- Human papillomavirus (HPV)-associated cancers are on the rise in the United States (CDC report).

- The most common HPV-associated cancers are oropharyngeal squamous cell carcinomas and cervical carcinomas.

- Virally-induced cancers could be more sensitive to immune modulatory treatments.

Immune checkpoint inhibitors do not demonstrate activity as single agents in the HPV-associated cancer model.
Combination Study – Immune Checkpoint Inhibitors Combined with Bifidobacteria Blend and an Epigenetic Immunomodulator

Study Design

- Determine baseline microbiome by 16S rRNA sequencing
- Establish tumors subcutaneously
- Administer Bifidobacteria Blend (BIF) 3 days prior to test agent administration and continue BIF supplementation for a total of 14 days

Treatment regimen

- Isotype Control ± BIF
- CTLA-4 inhibitor and PD-L1 inhibitor ±BIF
- Epigenetic Immunomodulator (EIM) ± BIF
- CTLA-4 inhibitor and PD-L1 inhibitor and EIM ± BIF

Measure TGI, survival, and changes in tumor infiltrating lymphocytes
In Vivo Efficacy

**Checkpoint Blockade + EIM ± BIF**

![Graph showing tumor volume over days for different treatment groups.]

**Pseudo-Survival Analyses**

![Graph showing percentage survival over study days for different treatment groups.]

- Isotype Control + Vehicle Control
- Isotype Control + Vehicle Control + Bacteria
- Isotype Control + EIM
- Isotype Control + EIM + Bacteria
- CTLA-4 Inhibitor + PD-L1 Inhibitor + EIM
- CTLA-4 Inhibitor + PD-L1 Inhibitor + EIM + Bacteria
- CTLA-4 Inhibitor + PD-L1 Inhibitor + EIM + Bacteria
Tumor Immune Cell Changes

Cytotoxic T-Lymphocytes

Granulocytes

% CD45+ Cells

% CD11b+ Cells
Tumor Immune Cell Changes

CD45+ Lymphocytes

CD4+ T-Cells
Ongoing/Upcoming Studies

- PD-1 ± BIF ± EIM in orthotopic murine pancreatic tumor model
- Expansion to assess the affects of microbiome manipulation on immune checkpoint inhibitor and agonist response in various models
Study Conclusions

► Mice acquire a stable TD2 microbiome after 14 days in our vivarium
  • TD2 offers syngeneic immuno-oncology models with stable and known microbiota regardless of the mouse vendor

► Supplementation with a Bifidobacteria formulation was well tolerated and associated with enhanced anti-tumor activity

► The HPV-associated syngeneic mouse tumor model is poorly immunogenic and does not respond to anti-PD-1 or anti-CTLA4 treatment
  • This could represent an ideal model to evaluate drugs that alter tumor immunogenicity and/or enhance the activity of checkpoint inhibitors

► Quantification of tumor infiltrating lymphocytes/immune effector cells using FACS demonstrates cellular changes that are associated with anti-tumor activity

► A novel small molecule epigenetic immunomodulator (EIM) compound improved the anticancer activity of immune checkpoint inhibition (anti-PD-1 + anti-PD-L1) that could be further enhanced by the concurrent administration of a Bifidobacteria formulation
  • Mechanistic studies are underway to better understand the contributions of the EIM to the improved immune checkpoint inhibitor activity and further enhancement by BIF
Preclinical Expertise: Designing and Conducting Clinically-Relevant Studies

► Personalized Preclinical support
  • Identification of the most relevant models for a given target to define sensitivity and resistance profiles for new agents
  • Application of clinical/regulatory context
  • Patient enrichment strategies integrated into every design
  • Combination strategies defined

► 250+ Established Models of Human & Murine Cancer
  • Validated In Vitro and In Vivo Growth Kinetics
  • Standard agent validation for combination strategies
  • Orthotopic models
  • Human and syngeneic models available
  • Expertise in Immuno-oncology models
    • Models with validated combination strategies for PD-1, PD-L1, CTLA-4, etc.
    • Melanoma, bladder, squamous lung, ovarian, pancreas, glioblastoma, etc.
    • Understanding of major checkpoint pathways

► Imaging and focal radiation equipment
► DMPK/Bioanalytical
Clinical and Scientific Leadership

Stephen Gately, PhD – President and CEO
- Obtained Ph.D. from McGill University in the Department of Neurology and Neurosurgery at the Montreal Neurological Institute and Hospital
- Assistant Professor Northwestern University Feinberg School of Medicine Division of Hematology/Oncology
- Searle/Monsanto (now Pfizer) – clinical development of novel inhibitors of angiogenesis for cancer
- Involved in the approval of celecoxib for familial adenomatous polyposis
- Inventor on more than 20 patents

Daniel Von Hoff, MD – Chief Development Officer
- Physician in Chief and Director of Translational Research at TGen (Translational Genomics Research Institute)
- Chief Scientific Officer for US Oncology (now McKesson Corporation) and for Honor Health's Clinical Research Institute.
- Clinical Professor of Medicine, University of Arizona
- Involved in the beginning of the development of many agents used routinely, including: mitoxantrone, fludarabine, paclitaxel, docetaxel, Abraxane, gemcitabine, irinotecan, nelarabine, capecitabine, lapatinib and others.