Translational Advances in Cancer Prevention Agent Development (TACPAD) – 2nd Biennial Virtual Meeting

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SPEAKER ABSTRACTS

PLENARY SESSION

Intercepting Pancreatic Cancer Development with oncogene Targeted Immunotherapy

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Pancreatic adenocarcinoma (PDAC) progression is triggered by a complex interaction of genetic mutations, stromal cell interactions and tumor microenvironmental (TME) signals. Diagnosis usually occurs late in disease progression, making treatment challenging and survival rates extremely poor. PDACs are also considered non-immunogenic, therefore newly emerging immunotherapies that have been successful in other cancers have not significantly progressed PDAC treatment options. Resistance to immunotherapies progresses as normal cells undergo the earliest genetic changes that transform them into early pre-malignant lesions. The two most common premalignant lesions are pancreatic intra-epithelial neoplasms (PanINs) and intraductal papillary mucinous neoplasm (IPMN). Both types of premalignant lesions eventually accumulate additional genetic changes that lead to early-stage invasive cancer and eventually, to late stage PDAC. This transformation process is influenced by both tumor-intrinsic and extrinsic forces within the developing TME. Accumulating data suggests that immune resistance mechanisms also evolve with this progression, which has led to the hypothesis that early immune intervention may be the best time to intervene to slow or even halt disease progression and improve treatment outcomes.

We know that tumor initiation to metastases takes years to decades, providing a unique window of opportunity for the **prevention** of premalignant progression. *KRAS* mutations are an early oncogenic event present in over 90% of PanINs and 75% of IPMNs. Initial studies examining cancer vaccines targeted to early oncogenic mutations are showing promise in animal models. A listeria-based vaccine engineered to express oncogenic $Kras^{G12D}$ combined with and without regulatory T cell depletion by an anti-CD25 antibody (PC61) and cyclophosphamide showed increased T cell infiltration, decreased disease progression, and increased survival in Kras-driven and p53 mutated genetically engineered mice ($Kras^{G12D/+}Trp53^{R172H/+}$;Pdx-1-Cre (KPC) mice) with early PanIN lesions but not those with later stage PanINs (Keenan et. al. 2014. Gastroenterology 146: 1784). Other early oncogenic mutations in PDACs may prove to be therapeutic targets for vaccine development.

We recently initiated a pilot study to examine the feasibility and safety of targeting mKRAS in patients with resected PDAC. For this, we developed a clinical–grade pooled **peptide vaccine** targeting 6 common *KRAS* mutations. This vaccine has been tested in combination with checkpoint blockade in 10 PDAC patients who had undergone surgery plus peri–operative chemotherapy and had remained disease–free (NCT04117087). Longitudinal immune phenotyping showed a robust peripheral mKRAS–specific T cell response against the most vaccinated epitopes. Flow cytometry revealed that these CD4 and CD8 T cells were activated, polyfunctional and displayed a memory phenotype. Based on the favorable immunogenicity and safety profile of our peptide vaccine in patients with resected PDAC, we have initiated a **Prevention Study** testing our mKRAS vaccine in individuals at high risk for PDAC. This study is currently enrolling and early results will be described.

Session I

Advances in Small Molecule Agent Development Pipelines: Promising Leads

Combination of Naproxen and a Novel Chemically-Stable Eicosapentaenoic Acid Analogue Provide Synergistic Tumor Protection in Pirc rats

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Abstract

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. Patients with the genetic disorder Familial Adenomatous Polyposis (FAP) develop hundreds to thousands of polyps that unless removed by prophylactic colectomy will progress to CRC at an early age. Non-steroidal anti-inflammatory drugs (NSAIDs) and ω-3 marine fish oils, such as eicosapentaenoic acid (EPA), have been evaluated for their chemopreventive potential in delaying the onset of CRC in high-risk patients. In this study, we determined whether the NSAID, naproxen, alone or in combination with a chemically-stable form of EPA (TP-252), affects tumor formation in the *Apc*^{Pirc} rat model. Rats fed a combination of naproxen and TP-252 exhibited a 95% reduction in tumor formation and a 98% reduction in tumor volume, respectively. To elucidate potential mechanisms of tumor protection, a comprehensive lipidomic analysis was performed on colonic mucosa to determine changes in eicosanoid metabolism. Animals receiving TP-252 alone or in combination with naproxen had significantly reduced mucosal levels of pro-inflammatory ω-6 eicosanoids (PGE_2, 5-HETE, and 14,15-DiHETrE), along with a simultaneous increase in anti-inflammatory EPA-derived ω-3 eicosanoids. Our colonic mucosal lipidomic analysis also uncovered several potential pharmacodynamic (PD) lipid biomarkers, including resolvin E2, 9-HEPE, 12-HEPE and 18-HEPE, that were increased in both the tissue and plasma of rats receiving TP-252 and were significantly correlated with tumor protection. Further studies with this drug combination should be focused on dose optimization and the role of EPA-derived lipid mediators in CRC initiation and progression.

SC144 and the next generation IL6/GP130/STAT3 inhibitors for cancer prevention

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IL6/GP130/STAT3 signaling plays a crucial role in multiple diseases, and all components of this signaling cascade are directly or indirectly implicated in tumorigenesis. Therefore, blockade of this signaling axis is expected to provide novel therapeutic opportunities to treat various diseases including cancer. IL6 binds to IL6R and GP130 to activate downstream signaling pathways to promote proliferation, survival and metastasis of cancer cells, and to stimulate angiogenesis on endothelial cells. Although GP130 is positioned at the junction of this oncogenic signaling network and is essential for activation of the network, there are no small-molecule inhibitors of GP130 under clinical development. Previously, we identified SC144 as a first-in-class, efficacious, safe, and orally active inhibitor of GP130. SC144 selectively inhibits the activation of downstream signaling pathways induced by GP130 ligands (IL6, LIF), with no significant effects on the activation by non-GP130 ligands, such as IFN-γ, SDF-1α, and PDGF. SC144 exhibits cytotoxicity in a panel of platinum-sensitive and platinum-resistant cells, with no significant cytotoxicity to human normal epithelial cells. In mouse xenograft models, SC144 significantly inhibited tumor growth through GP130 inhibition and induction of necrosis in the tumor. No toxicity was evident in normal tissues. Furthermore, SC144 showed significant *in vivo* efficacy in immune competent syngeneic mouse models. Unfortunately, clinical development of SC144 was delayed due to its poor solubility and metabolic instability. During the past two years, we have identified metabolic liabilities of SC144 and have undertaken an extensive medicinal chemistry lead optimization campaign to produce analogs with increased solubility and metabolic stability. The new analogs show favorable properties in single- and repeat-dosing pharmacokinetic (PK) studies. As a result, we have in hand a series of 2nd-generation analogs that are orally active, water-soluble, and display desirable PK properties suitable for advanced preclinical studies to support IND filing.

Targeting STAT3 with TTI-101, an Oral Small Molecule, to Prevent Colorectal and Hepatocellular Cancer

Prema Robinson; Leticia Hamana; Laura Beretta; Moses Kasembeli; Uddalak Bharadwaj; Yun Shin Chun; Yinghong Wang; Xeumei Wang; Laura Reyes; Luisa Solis; Asif Rashid; Dipen Maru; Eduardo Vila; Apostolia Tsimberidou; Ahmed Kaseb; Scott Kopetz; and David Tweardy. University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Persistent STAT3 activation contributes to 10 of 14 hallmarks of cancer, including inflammation; successful targeting of STAT3 has the potential to prevent and/or treat cancer. However, no small molecule that directly targets STAT3 has been FDA approved. The Tweardy lab used computer-based docking of drug-like compounds into the SH2 domain of STAT3, along with hit-to-lead optimization and medicinal chemistry, to identify TTI-101; TTI-101 treatment was safe, hit its target in tumor cells, and resulted in clinical benefit in a Phase I trial of patients with advanced solid tumors.

The incidence of colorectal cancer (CRC) is increased 20-30 fold in patients with inflammatory bowel disease (IBD), while 90% of hepatocellular carcinomas (HCC) arise in the setting of chronic inflammation. To assess the contribution of STAT3 to CRC secondary to IBD and to HCC, we performed immunohistochemistry (IHC) staining and computer-based scoring for activated STAT3 (phosphorylated on Y705, pY-STAT3) of epithelial and stromal cells within colonic endoscopic biopsies and surgically resected CRC from IBD patients, as well as of tumor cells and hepatocytes within surgically resected HCC tumors. Compared to epithelium of normal tissue, levels of pY-STAT3 were increased 1.9-fold in dysplastic epithelium ($p=0.05$) and 1.8-fold in the stroma of normal tissue ($p<0.0001$). In surgically resected HCC tumors, lower pY-STAT3 scores in tumor cells, but not hepatocytes, correlated with longer recurrence free survival (RFS; $p=0.003$).

TTI-101 administration in three AOM-DSS mouse models of IBD resulted in a dose-dependent reduction in polyps, adenomas, and/or adenocarcinomas. TTI-101 administration to the Hep*Pten*$^{-}$ mouse model of NASH-induced HCC resulted in a dose-dependent reduction in liver pY-STAT3 levels. Thus, STAT3 may be a valid target for chemoprevention using TTI-101 in CRC arising from IBD and in HCC.

We thank Tvardi Therapeutics for providing TTI-101 for these studies.

Discovery and Development of LFA-9 for CRC Prevention

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Colorectal Cancer (CRC) is a major public health issue with an estimated 980,000 deaths annually, worldwide. Current trends show an increase of CRC incidence and mortality worldwide. Colonic adenomas are very common in ages 50 and over. Though, nonsteroidal antiinflammatory agents like Aspirin, Naproxen and COX-2 inhibitor and Celecoxib are useful to prevent the polyp progression and reducing CRC burden, continuous/chronic usage of these drugs are limited by GI toxicity and unwanted side effects. Thus, the rationale to establish safer anti-inflammatory agents for CRC prevention is important. Mechanistic studies suggest that up-regulation of 15-PGDH, sparing COX-1/2 and prostaglandin (PG)I_2 synthase and/or selectively targeting mPGES-1 and 5-LOX would reduce the cardiovascular side effects and may improve the chemopreventive efficacy. There are limited to no studies on targeting mPGES-1/5LOX and it is one of the unexplored areas in colorectal cancer chemoprevention towards safer drug development. To design and develop selective inhibitors of dual mPGES-1/5-LOX, we used *in-silico* small molecular docking simulation approaches, and identified LFA-9 as a novel duel mPGES-1/5-LOX inhibitor among >35 analogs. Azoxymethane (AOM)-induced rat colonic tumors were utilized as ex-vivo to assess pharmacodynamic inhibitory effects of LFA-9 on mPGES-1 and 5-LOX by Radio-HPLC. In a series of animal experiments, we established the dose-range of toxicity and optimal doses of LFA-9. Potential preventive efficacy of LFA-9 was assessed AOM-rat colon carcinogenesis (Aberrant Crypt Foci, (ACF) as surrogate marker in male F344 rats and intestinal tumor inhibition in $APC^{Min/+}$ mice. Furthermore, we have shown the efficacy of LFA-9 administered in the diet at the adenoma stage significantly suppressed colonic adenocarcinoma formation in AOM-induced colon cancer (sporadic CRC model) and PIRC rat model (FAP model). Overall, the above results show that LFA-9, a novel dual mPGES-1/5-LOX inhibitor, is a safer agent and has potential for prevention of CRC in high-risk patients. {This work was supported by NCI-PREVENT Cancer Program, R01 CA213987 and VA Merit Awards}

Reverse-engineering discovery of targets, involved cell types, and compounds for liver cancer chemoprevention

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Hepatocellular carcinoma (HCC) is a cancer type with clinically unequivocal and well-defined predisposing conditions, namely chronic fibrotic liver diseases caused by viral hepatitis and metabolic disorders. By following up the at-risk patient cohort, natural history of HCC development can be captured over the timeframe of one to two decades. Archived biospecimens are often available from the time of initial liver disease diagnosis, and unbiased molecular profiling of the specimens provides unique opportunities to interrogate molecular dysregulations confidently correlated with the real long-term clinical outcome, i.e., HCC development from early-stage liver diseases. This “reverse-engineering” strategy helps increase the likelihood of identifying clinically relevant therapeutic targets without solely relying on data from experimental preclinical models. This is particularly useful in cancer chemoprevention target discovery given the the requirement of overwhelmingly lengthy and costly clinical validation of experimentally derived candidate targets, which is often practically infeasible. Utilizing this strategy, we could successfully identify HCC chemoprevention targets (e.g., cellular signaling such as EGFR pathway, circulating bioactive lipids), involved cell types (e.g., hepatic myofibroblasts, conventional dendritic cells), and compounds (e.g., small molecular signaling pathway inhibitors, generic agents), streamline the process of their clinical validation, and facilitate translation of promising chemoprevention agents to the care of liver disease patients.

Session II

Translation from Bench to Bedside: Challenges and Resources

PREVENT Agent Development Pipeline

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The NCI 's PREVENT Cancer Preclinical Drug Development Program is a peer-reviewed program designed to support the preclinical development of promising agents and biomarkers for cancer interception/prevention towards clinical applications. PREVENT is not a grant program but allocates NCI contract resources to advance approved projects in a milestone-driven manner. Results obtained through NCI contract resources are returned to the applicant PIs and used to support further development by the applicants or in partnership with NCI. Resources available to PREVENT Program applicants include preclinical efficacy testing, CGMP manufacturing, GLP pharmacokinetic and IND-enabling toxicology studies, and IND filings. The PREVENT Program is focused on preventive agent development in the areas of Immunoprevention (cancer vaccines and immunomodulatory agents), Chemoprevention (novel mechanisms, anti-inflammatory agents, drug repurposing, toxicity reduction via alternative dosing regimens and agent combinations) and clinically translatable mechanistic biomarkers (pharmacodynamics, immune correlates, and tumor preventive efficacy). Submission deadlines for PREVENT Concept Applications occur twice per year on the second Monday in January and July. Further information can be obtained at the PREVENT Program website:

https://prevention.cancer.gov/major-programs/prevent-cancer-preclinical

How to move a preventative agent from design into approval

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Cancer prevention is a global public health challenge that requires a multidisciplinary approach including biology, epidemiology, medicine, nutrition, education, clinical trials and public policy. An intent-to-treat analysis of a randomized controlled trial differs from that in the observational setting, therefore, it is important to understand the underlying disease process and aspects of the intervention through research. The genetic heterogeneity of some cancers strongly support an enhanced focus on prevention as a strategy in high-risk individuals. Important factors when developing preventative cancer agents include the time course of the intervention, dose and duration of exposure needed to effect risk reduction, trial endpoints, durability of the impact of intervention, and methodological problems in implementing and interpreting randomized trials to evaluate prevention strategies. The clinical development of cancer prevention therapeutics has many challenges, including the long duration necessary to conduct trials, the lack of adherence to therapy, and unanswered questions on the best endpoint remain. The government, whether it be local, state or national, plays a vital role in regulation and education. The history of chemoprevention regulatory approvals has taught us that these programs are important in the fight against cancer. The Food and Drug Administration has identified barriers to clinical trial participation and challenges in surrogate endpoints, although with a multidisciplinary approach including communication with industry, are extremely positive about the future direction of chemoprevention research.

National Cancer Institute Small Business Innovation Research Development Center
NCI SBIR & STTR Resources for Commercializing Innovative Cancer Prevention

The Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) Programs at the National Institutes of Health (NIH) serve as one of the largest sources of early-stage capital for technology commercialization in the United States (US). In FY2021, the National Cancer Institute (NCI) SBIR and STTR Programs provided approximately $182M in non-dilutive funding to US small businesses and managed over 400 different projects. By supporting startups and other small businesses in the cancer space, the NCI SBIR and STTR Programs act as catalysts of innovation for novel technologies and products to achieve NCI's mission to prevent, diagnose, and treat cancer. Staffed by a dedicated team of scientists with wide-ranging technology and industry experience, the NCI SBIR Development Center provides both funding and non-funding resources to US small businesses all aimed at translating technologies to the clinic to help cancer patients and providers.

During the past 5 years, NCI has provided SBIR/STTR funding to approximately 25 companies who are developing novel cancer prevention agents. Companies focused on prevention of several indications such as colorectal cancer, oral squamous cell carcinomas, and human papillomavirus-related cancers. Award sizes ranged from $200k-$2M and included support for companies with technologies in the preclinical and clinical stages of development. NCI provided opportunities for non-funding support as well, including entrepreneurial training programs such as I-Corps™ at NIH and programs to facilitate connections between innovators and potential investors. Whether a technology is in the preclinical or clinical stage of development, NCI can help small businesses translate their innovative research to the marketplace so they become available to healthcare providers and their patients.

NCATS Collaborative Translational Research/Drug Development Resources

The mission of the National Center for Advancing Translational Sciences (NCATS) is to catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of interventions across a wide range of human diseases and conditions. NCATS is directly addressing this problem by discovering new technologies and other approaches that could greatly accelerate the process of developing and deploying therapeutic solutions. An introduction of the Center's internal research operations will be presented, focusing on the use of advanced assay and chemistry technologies to address the wide range of needs associated with early discovery space. Further, an overview of collaborative opportunities for joint projects will be provided.

Session III

State of the Science and Advances in Immunomodulatory Agents Development

Modulation of tumor associated macrophages in breast cancer

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Macrophages are an innate immune cell that play a critical role in host defense and maintaining tissue homeostasis, however their infiltration into tumors has been associated with disease progression and resistance to therapy. Tumor associated macrophages (TAMs) represent a significant proportion of solid tumors, including breast cancer. TAMs play a major role in tumorigenesis as they can enhance tumor cell growth, angiogenesis and metastasis. In addition, TAMs can inhibit anti-tumor responses of T cells. Our recent work has shown that removal or conversion of TAMs to an anti-tumor phenotype enhances chemo- and immuno-therapy establishing TAMs as targets for anti-cancer therapy. We recently revealed that a Class II HDAC inhibitor can polarize TAMs to an anti-tumor phenotype to facilitate reduction in primary and metastatic tumor burden. In addition, we have found that some types of therapy such as poly(ADP-ribose) polymerase (PARP) inhibition (PARPi) can drive development of highly suppressive TAMs, restricting anti-tumor T cell function and survival. Murine models demonstrate that in the absence of TAMs, PARPi induce a robust recruitment of cytotoxic T cells and durable antitumor responses. Therefore, targeting TAMs is a promising strategy for improving PARPi treatment efficacy. However, while there is an urgent need to target TAMs during tumorigenesis and cancer therapy, to date, failure to fully characterize TAM biology and classify multiple subsets has hindered advancement in therapeutic targeting. Deep analysis of TAMs in solid tumors has revealed the complexity of TAMs and revealed major gaps in our knowledge of the functional and phenotypic characterization of TAM subsets associated with cancer, before and after treatment. Here we will discuss the complexity of TAMs in solid tumors including characterizing TAM subsets, location, and crosstalk with neighboring cells, as well as novel TAM-modulating strategies and combinations that are likely to enhance current therapies and overcome chemo- and immuno-therapy resistance.

Sulindac modulates the response of proficient MMR colorectal cancer to immune checkpoint inhibitors

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Immune checkpoint inhibitors (ICIs) therapy has been widely used to treat different human cancers, particularly advanced solid tumors. However, clinical studies have reported that ICI immunotherapy benefits only ~15% of colorectal cancer (CRC) patients, specifically those with tumors characterized by microsatellite instability (MSI), a molecular marker of defective DNA mismatch repair (dMMR). For the majority of CRC patients who carry proficient MMR (pMMR), ICIs have shown little clinical benefit. In this study, we examined the efficacy of sulindac to enhance the response of pMMR CRC to anti-PD-L1 immunotherapy. We first utilized CT26 and CMT93 syngeneic mouse tumor models to compare the inhibitory effects of PD-L1 antibody (Ab), sulindac, and their combination on pMMR CRC tumor growth. We found that mice treated with combination therapy showed a significant reduction in tumor volume, along with increased infiltration of CD8+ T lymphocytes in the tumor tissues. We also demonstrated that sulindac could downregulate PD-L1 by blocking NF-κB signaling, which in turn led to a decrease in exosomal PD-L1. Notably, PD-L1 Ab can be bound and consumed by exosomal PD-L1 in the blood circulation. Therefore, in combination therapy, sulindac downregulating PD-L1 leads to increased availability of PD-L1 Ab, which potentially improves the overall efficacy of anti-PD-L1 therapy. We validated all these results using humanized Patient-Derived Xenografts (PDX) animal models. Moreover, we demonstrated the safety of low-dose sulindac as it did not have a systemic inhibitory effect on prostaglandin E2 (PGE2). In conclusion, our findings provide unique insights into the mechanism of action and efficacy for sulindac as an immunomodulatory agent for the ICI therapy and immunoprevention of pMMR CRC.

Preclinical testing of CD73 inhibitors for pancreatic cancer immunoprevention

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Introduction: The all stages combined five-year survival rate for pancreatic adenocarcinoma (PDA) is 11%; however, the five-year survival rate for localized PDA is 42%. These statistics highlight the importance of early prevention strategies to prevent disease progression and metastatic dissemination. Through the NCI PREVENT program, this research program explores immunoprevention strategies for PDA by targeting CD73, a gatekeeper ectoenzyme responsible for production of extracellular adenosine. We have recently shown aggressive subtypes of pancreatic intraepithelial neoplasia (PanIN) and PDA arising in ductal pancreatic epithelium have elevated CD73 and intrapancreatic adenosine indicating adenosine generation may be an early trigger of immunosuppression. We hypothesize inhibition of CD73 and adenosine generation will promote a more robust anti-tumor immune response and prevent PanIN and PDA progression.

Methods: We tested three small molecule CD73 inhibitors (APCP, OP-5244, and AB680) in a syngeneic PDA mouse model by injecting 100-200k murine PDA cells derived from *Kras^{G12D};Trp53$^{R172H/+}$;Pdx:Cre* (KPC) mice in the flanks of C57BL/6 female mice. Tumor sizes were measured weekly and tumor volume and mass were recorded at time of death. Dosage: APCP oral gavage (3x/week at 20mg/kg) and intraperitoneal (IP) (3x/week at 20 mg/kg). OP-5244 oral (3x/week at 25mg/kg and 10mg/kg). AB680 oral gavage (3x/week at 10mg/kg). HPLC analysis was performed for each inhibitor to quantify adenosine levels.

Results: IP delivery of APCP significantly reduced tumor growth and intratumoral adenosine levels; however oral gavage delivery did not reduce tumor growth. Similarly, oral gavage delivery of OP-5244 did not reduce tumor growth. AB680 significantly reduced tumor volume and intratumoral adenosine levels and CyTOF immunoprofiling showed activated CD8+ T cells, dendritic cells, and macrophages were significantly increased in the tumors from AB680 treated mice.

Conclusion: APCP IP delivery is more effective than oral gavage delivery and OP-5244 oral gavage delivery does not significantly decrease tumor growth. AB680 oral gavage delivery significantly decreases tumor growth and tumor adenosine concentrations. We observed a significant increase in infiltration of activated CD8+ T cells. AB680 shows high translational potential for preclinical testing in spontaneous GEM models.

Session IV

Emerging Vaccines for Cancer Prevention

Mass Spectrometry and RNAseq Based Discovery of Frameshift Neoantigens and Cryptic Neoantigens from Malignant Peripheral Nerve Sheath Tumors for Prophylactic Vaccines

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Neurofibromatosis type 1 (NF1) syndrome is an autosomal, dominant tumor predisposition syndrome caused by loss-of-function mutations of *NF1* gene encoding neurofibromin. Loss of the non-mutant allele of *NF1* in a rare Schwann cell or precursor leads to benign plexiform neurofibromas. The main cause of death among NF1 patients is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive soft tissue sarcoma that most likely develops from plexiform neurofibroma, in particular the so-called “atypical” plexiform neurofibroma. Approximately half of MPNSTs are NF1-associated, and *NF1 patients have ~15% lifetime risk of developing this terrible cancer*. NF1 patients could greatly benefit from prophylactic vaccination that would prevent the malignant transformation to MPNSTs. We hypothesize that malignant transformation leads to the expression of recurrent alternately processed transcripts, such as transcriptionally-induced chimeras, that could express neoantigens and be used as targets for prophylactic vaccines. Such transcripts can be translated to produce novel peptides downstream of frameshift mutations caused by coding exon read-through into introns, mis-splicing from a coding exon to a non-canonical splice acceptors or splice acceptors in other genes. In most cases, a premature termination codon (PTC) will be rapidly encountered by the ribosome translating such transcripts. Therefore, we furthermore hypothesize that these alternately processed transcripts can express what we call “cryptic” neoantigens when treated with drugs that suppress utilization of premature codons such as Ataluren or gentamicin. In such a way, we could administer a prophylactic vaccine and induce conditionally active immune response that would eliminate nascent tumors only when drug treatment is used. We have developed a PTC suppression read-through reporter which results in presentation of the SIINFEKL peptide on C57BL6/J tumor cells. We developed a bioinformatic algorithm that takes deep RNA-seq data and identifies candidate neoepitopes encoded by non-normally processed or mutant mRNAs called *Identifying and Verifying Immunogenic Neoepitopes and Neojunctions* (IVINN). We have also achieved successful purification of human HLA Class I complex using an anti-pan HLA antibody column, and bound peptides have been isolated and identified by liquid chromatography tandem mass spectrometry (LC-MS/MS). We plan to utilize these approaches for mouse model and human MPNST patient derived xenografts.

Immunoprevention of Triple Negative Breast Cancer by TOP2A Derived Peptide Vaccination

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Top2A is a key enzyme involved in DNA replication and is a therapeutic target for several cancer types including breast cancer. Overexpression of Top2A has been observed in both human and mouse triple-negative breast cancer (TNBC). The present study evaluated both immunogenicity and antitumor efficacy of a newly formulated multi-peptide vaccine targeting multiple epitopes of the Top2A protein. Top2A-specific MHC II epitopes with optimal binding affinity were identified using a combined scoring system, which predicted their potential to elicit a Th1 immune response. The formulated vaccine contained top three Top2A peptides, which elicited the strongest immunologic response and showed 100% sequence homology between human and mouse. Anti-tumor efficacy of the Top2A vaccine was initially evaluated in a syngeneic TNBC mouse model, in which pre-graft preventive vaccination was associated with significantly decreased tumor growth as compared to the adjuvant controls. The Top2A peptide vaccine exhibited striking efficacy in a genetically engineered TNBC mouse model (C3(1)/Tag), reducing tumor burden by >90% when compared with adjuvant alone. Splenocytes collected from vaccinated animals showed a robust immunologic response to the immunizing peptides. There were no overt toxicities observed with the Top2A vaccination. To explore potential mechanisms underlying the anti-tumor response induced by Top2A vaccine treatment, scTCR-seq of tumors in both control and Top2A vaccine groups revealed new T cell clones as a consequence of Top2A vaccination. Furthermore, *in vitro* stimulation of these splenocytes by the vaccinated Top2A peptides resulted in the secretion of cytokines indicative of Th1 responses but with minimal secretion of Th2-related cytokines. Our data indicate that the newly developed multi-peptide Top2A vaccine is immunogenic and efficacious in the prevention of TNBC development and progression *in vivo*.

Lessons learned from clinical trials of MUC1 peptide vaccines for cancer prevention

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We report on three trials of a MUC1 peptide vaccine for cancer prevention. Two trials were pilot studies evaluating vaccine immunogenicity and safety. One was in subjects with advanced colon adenomas, and the other in smokers at risk for lung cancer. The third trial was a multi-center, randomized placebo-controlled trial in subjects with advanced adenoma that included an assessment of the clinical efficacy of the vaccine to prevent recurrent adenoma. All trials successfully recruited their full complement of participants and the vaccine was well tolerated with no safety concerns. The response rate in the colon adenoma trials was 43% in the pilot study and 25% in the placebo-controlled multicenter trial. Only 10% of smokers responded to the vaccine. Higher levels of circulating myeloid derived suppressor cells (MDSC) were consistently associated with the lack of an immune response. This suggests that even in pre-malignancy immunosuppressive tendencies can impair vaccine immunogenicity. Responders to the vaccine demonstrated immune memory at one year. In the multicenter adenoma trial that evaluated the efficacy of the vaccine on adenoma recurrence, immune responders demonstrated an association with a reduced rate of adenoma recurrence. In future studies, patient selection based on circulating MDSC levels, or concomitant use of agents to counter MDSC immunosuppressive function or otherwise improve T cell function, should be considered.

Session V

Cancer Prevention Clinical Trials

Iloprost for Lung Cancer Prevention

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Iloprost is a prostacyclin analogue with anti-metastatic, vasodilatory and anti-inflammatory properties. Increased prostacyclin is protective in pre-clinical murine models of adenocarcinoma and squamous cell carcinoma (SCC), including tobacco smoke. The chemoprotective properties are independent of the single cell surface prostacyclin receptor, as knockout animals are still protected. Clinical studies of iloprost have included both oral and inhaled preparations. Oral iloprost improves endobronchial dysplasia (pre-cursor lesions for invasive SCC) in former smokers. Dysplastic lesions are now being characterized beyond histology to better understand lesion evolution. Investigations into the mechanism of iloprost associated cancer prevention (including effects on basal progenitor cells, progenitor multi-potentiality, and differentiation) and biomarkers predicting response will be presented, along with an outline for an upcoming clinical trial in former smokers.

Cancer Immune-Interception in Lynch Syndrome: Neoantigen-based Vaccine Development

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Lynch Syndrome (LS) is the most common cause of hereditary colorectal cancer and also conveys significantly increased risks for several other malignancies, including endometrial, small bowel, gastric, ovarian, adrenocortical tumors, and others. LS results from a heterozygous germline mutation in one of four DNA mismatch repair (MMR) genes. The acquisition of a somatic “second hit” causes affected cells to lose the ability to repair DNA mismatch replication errors that frequently occur in microsatellite regions of the genome. Consequently, hundreds to thousands of small insertion/deletions (indels) accumulate in these regions, and when expressed, result in frameshift peptides that are recognized as neoantigens that are then presented on the cell surface via the major histocompatibility complexes (MHC-I/II) for immune cell recognition. Vaccine development efforts in this high-risk population have been challenged by the substantial inter-individual variability in both the set of expressed neoantigens and MHC I/II responses.Yet, recent advancesin next-generation sequencing and associated bioinformatic approaches are now allowing for more accurate profiling of the most frequently recurring and shared mutated neoantigens in LS-associated tumors. This allows for identification of the most immunogenic neoantigens that can be incorporated into different vaccine platforms to test the development of a population-based vaccine. The Vilar Lab has collaborated with industry and academic partners such as Nouscom, s.r.l. and the National Cancer Institute to develop two different clinical trials to bring new vaccines to the LS population based on mutated neoantigens and tumor associated antigens. In this presentation, we will focus on mutated neoantigen-based strategies and present the Phase I clinical trial (NCT05078866) using a viral-based vaccine including 209 distinct neoantigens. The primary endpoint of this trial is safety and immunogenicity. A total of 45 participants will be enrolled to receive a prime and boost vaccination. Different secondary biomarkers will explore immunological aspects of this novel immune-interception strategy.

Clinical trials of GUCY2C (GCC) agonists for colorectal cancer prevention.

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Guanylyl cyclase C (GUCY2C), expressed on intestinal epithelial cells, is the receptor for heat-stable enterotoxins (STs) produced by diarrheagenic bacteria. Fluid secretion reflects ST activation of GUCY2C, which produces cyclic (c)GMP activating ion channels. Beyond toxins, GUCY2C binds uroguanylin in small intestine and guanylin in colorectum, which also stimulate secretion. This mechanism is the basis for the oral agonists linaclotide (ST analog) and plecanatide (uroguanylin analog) to treat constipation. Guanylin is the most commonly lost gene product, while GUCY2C expression is retained, in colorectal tumors. Eliminating GUCY2C promotes tumor formation, while oral GUCY2C agonists or transgenic guanylin reduce intestinal tumors, in mice. These data support a hypothesis in which loss of guanylin silencing GUCY2C is a key step in intestinal transformation which can be prevented by oral GUCY2C agonists. However, GUCY2C agonists are formulated for duodenal activity, without bioavailability in the colorectum. Here, we explored whether this pharmacokinetic (PK) barrier could be abrogated with high doses (HD) of linaclotide. Indeed, HD linaclotide induced a cGMP response in mucosal biopsies obtained by ***colonoscopy*** following oral polyethylene glycol (PEG) preparation. This cGMP response was validated by VASP phosphorylation and suppression of crypt proliferation. Similarly, HD linaclotide induced cGMP in colon biopsies obtained by ***sigmoidoscopy*** following oral PEG. However, HD linaclotide failed to induce cGMP in colon biopsies obtained by sigmoidoscopy ***in the absence of oral PEG***. Thus, oral PEG abrogated the PK barrier, delivering linaclotide to the colorectum. Unfortunately, dolcanatide, a proteolysis-resistant analog of plecanatide, also failed to overcome the colorectal PK barrier, although it produced duodenal secretion and diarrhea. Thus, guanylin loss silencing GUCY2C appears to be a key step in intestinal transformation. Preclinical models suggest that oral GUCY2C agonists stimulate GUCY2C signaling, opposing tumorigenesis. However, translation of these observations appears to be constrained by a PK barrier, reflecting agonists formulated for duodenal activity, without colorectal bioavailability. Exploiting GUCY2C for chemoprevention awaits agonists formulated for the colorectum. We are exploring the ability of GUCY2C agonists to activate cGMP in duodenum (within the PK barrier), as a prelude to trials in patients with FAP at risk for duodenal tumors.

Targeting mTOR signaling in oral premalignant lesions: From bench to clinic and back

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Despite encouraging recent results from novel treatment options, such as immunotherapy, for head and neck squamous cell carcinoma (HNSCC), limited progress has been made in improving outcomes for most patients. Prevention and early detection are key to improving the prognosis of HNSCC. Our team has focused on decoding the oncogenic signaling circuitries driving HNSCC initiation and progression, aimed at identifying novel druggable targets to treat and prevent this aggressive malignancy. These efforts led to the early discovery that persistent activation of PI3K/mTOR signaling circuitry is the most frequent dysregulated signaling mechanism in HNSCC, and that in turn, the overreliance on PI3K/mTOR for HNSCC initiation and progression can be exploited for therapeutic purposes. Evidence will presented that mTOR inhibition exerts a potent antitumor activity in HNSCC patients in a recently reported window of opportunity clinical trial (NCT01195922). Thus, mTOR inhibitors can be considered for the prevention of HNSCC development and for the treatment of existing HNSCC lesions. However, their safety profile and tolerability may hamper their potential long-term use for HNSCC prevention. In this regard, we have shown that the repurposed drug metformin, which is safely used by millions of type 2 diabetes patients, decreases mTOR signaling in HNSCC and displays potent chemopreventive activity in experimental oral premalignancy models. Based on these findings, we have conducted a Phase IIa Clinical Trial using metformin for HNSCC prevention (NCT02581137) in patients with oral premalignant lesions (OPL), which was recently completed. Metformin administration resulted in mTOR inhibition, and improvement in the histological severity of 60% of the OPLs, including a subset (17%) of patients that exhibited complete responses. Ongoing experimental and planned clinical studies will be presented, which may provide a mechanistic framework for the use of metformin as a precision preventive agent for HNSCC.