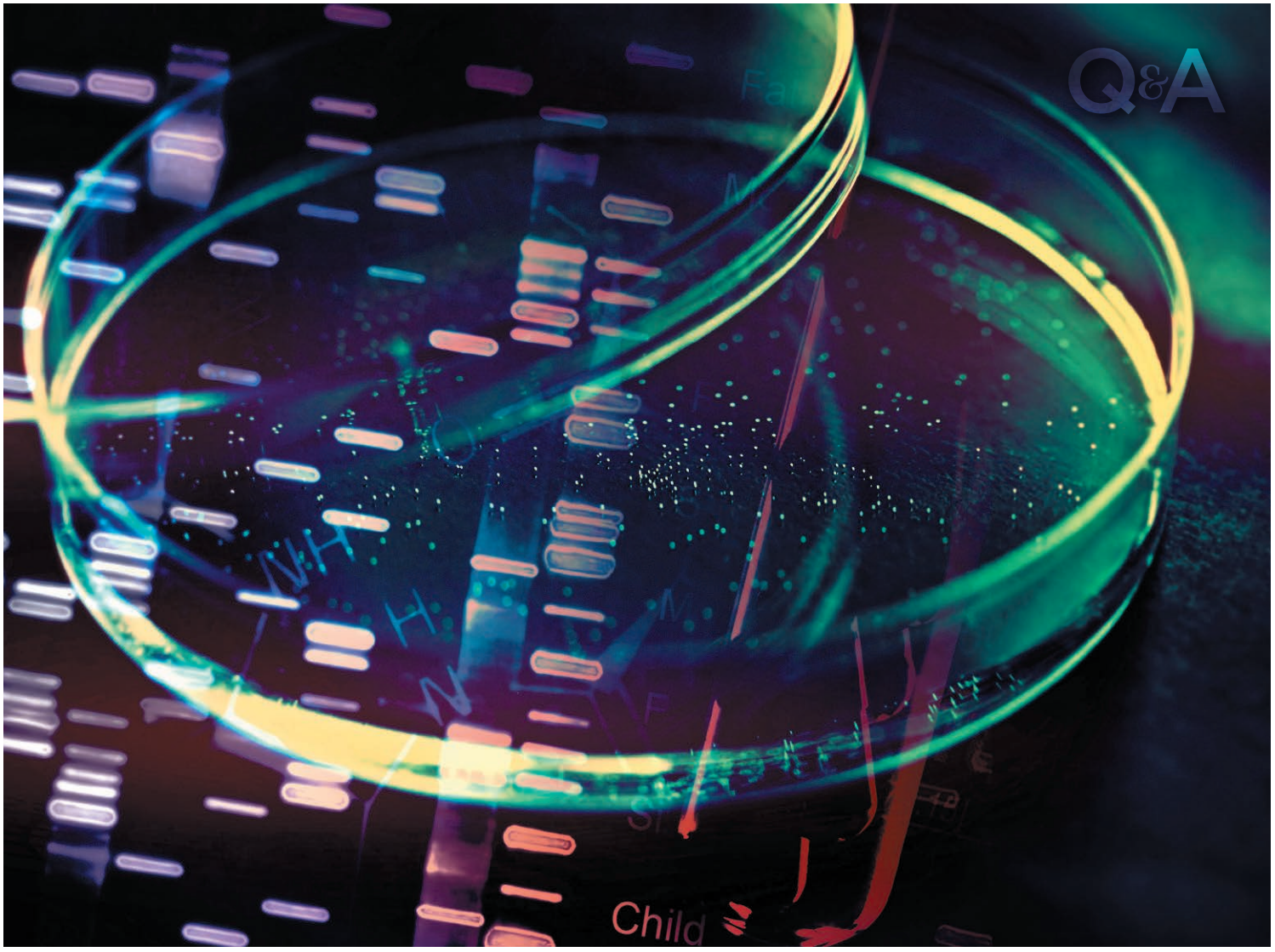

Research at Cedars Sinai – CANCER

An interview with Dan Theodorescu

WHILE SEARCHING online for early pioneers in precision medicine, we happened upon Dan Theodorescu, MD PhD. Dr Theodorescu is the Director of the Samuel Oschin Comprehensive Cancer Institute and overall leader of the Cedars-Sinai Cancer Program. Cedars-Sinai CANCER has been a top 10 ranked cancer program in the Unites States according to *US News and World Report* since 2020. He is also the Principal Investigatort of the Convergence Science Virtual Cancer Center (csvcc.org). From 2010 until 2018, he was Director of the University of Colorado Cancer Center and a Distinguished University Professor. (See Dan's biography for more detail.)

We asked a series of questions about biomarkers in cancer, their role in tracking disease progression, their use in diagnosis, and how they might lead to precise medicines. We also ask about two recent programs that leverage Theodorescu's research and educational passions.

Q1. For background, as a urologist, you selected bladder cancer as a model system to study cancers. What led you to use bladder cancer to search for genes that might be implicated in cancers in other tissues?

A. I have wanted to do Cancer Research ever since I was 10 or 11. Later, as I was reading about cancer while in college, I became fascinated with bladder cancer. It seemed to me that bladder cancer was

not only an important public health problem that led to a lot of suffering, but also a disease that was very amenable to molecular and cellular investigation with the hope to use that knowledge for developing novel therapeutics. One of the turning points in my career that firmly led me to embark on studying bladder cancer was a meeting that I attended soon after starting grad school in which Bert Vogelstein gave a phenomenal talk on the stepwise development of colon cancer. It seemed to me that bladder cancer offered the same opportunities to investigate biology in a similar organ, namely aluminal organ, sloughed cancer cells in this urine, that was bathed by a fluid (in this case urine) that could be examined at the molecular level. The other great advantage of bladder cancer was the fact that it was easily accessible by minimally invasive technology such as cystoscopy and biopsy. These features led me for the last 30 years to study this disease with great passion and satisfaction.

However, as molecular understanding of cancer evolved, it became clear that some of the genes involved in bladder cancer biology were also potentially relevant in other cancers. This led me progressively look at some of the genes or therapeutics that we found relevant in bladder in other cancer types. In fact, while the GTPases are important in bladder cancer, the animal models used in evaluating our Ral GTPase inhibitor were lung cancer and pancreatic cancer, as those cancers would be the prime diseases in which early clinical trials would be carried out to test any potential drug. In addition, we also examined the concept that cancers that have an important and shared etiologic agent (such as smoking) would perhaps have common biomarkers that could be used for precision prognostication. We examine this in bladder cancer and lung cancer and indeed found such commonalities do exist. This was very exciting and related to the concept I allude to below, namely that the molecular circuitry/architecture of the cancer cell is perhaps more important than the tissue of origin.

Q2. Among the genes you have studied, RhoGDI2 is of interest as it seems to play a role in tumor suppression in bladder cancer metastasis but may result in tumor progression when overexpressed.

Could you discuss briefly the impact of over vs under-expression levels of RhoGDI2 in bladder tissue?

Does that same analysis extend to other tissues? Or is the behavior of RhoGDI2 "agnostic" to the tissue type?

A. Yes, this is indeed true and very interesting. While we discovered RhoGDI2 using transcriptional profiling and computational methods of bladder cancer and validated its role as a metastasis suppressor using both human tissues and clinical outcomes as well as experimental studies, it has been clear that in other tumor types RhoGDI2 acts as a tumor driver. The exact reason for this is still unclear but it is not unique to RhoGDI2 and indeed highlights the importance of the cellular context that is specific gene abnormality manifests. And I say this specifically as cellular context or molecular architecture as opposed to tissue type or origin is likely more important in determining how a cancer cell will behave in response to any one specific abnormality. This does not mean, however, that tissue type is not an important variable, but it is probably less than the overall molecular architecture of the cell and all its abnormalities.

"There is no doubt that immunotherapy for cancer using checkpoint inhibitors has been a revolutionary advance"

Q3. On the topic of novel approaches to precision cancer medicine, could you please provide a brief description of drug development project that arose from your work with genes that promote invasion and metastasis?

A. We had been studying for years the molecular biology underpinning bladder cancer invasion and metastasis. It became clear from panomic investigations (especially transcriptional profiling) of bladder cancer cell lines and human samples that the GTPase family of molecules were very important in this process. For a variety of reasons we focused on Ral GTPase as a major player in tumor progression and growth. The Ral GTPase was part of a family of this relatively undruggable molecule GTPase family.

Despite some trepidation given the "undruggability" of GTPases, we set out to try and discover new inhibitors to Ral. The reason why we attempted this is that we had the novel idea that instead of trying to attack the active form of the Ral GTPase molecule as many have in the past for other GTPases, we would instead target the inactive form of the molecule. This took advantage of the continuous activation-deactivation cycle that all non-mutated GTPases undergo; therefore, by trapping a molecule in its inactive form, the pool of active molecules is eventually depleted. Using computational and biochemical methods we are able to identify suitable inhibitors and a new pocket on the Ral GTPase where this inhibiter could lodge. In addition this approach was proof of principle that identification of pockets on molecules that are only present in the inactive form that could be used therapeutically to trap molecules in this form is feasible is it feasible way to inhibit the molecular activation.

Q4. On Discovery of Tumor and Metastasis Regulator Genes: In addition to RhoGDI2, you have also investigated he CCL2-CCR2 signaling axis in cancer metastasis and showed that inhibition of the CCL2 receptor, CCR2, both reduces metastasis and enhances tumor response to immune checkpoint therapy. Can you discuss the impact of your work on immunotherapies?

A. There is no doubt that immunotherapy for cancer using checkpoint inhibitors has been a revolutionary advance. Despite this advance, there is still progress to be made as we are not curing every single patient with advanced epithelial cancers with this therapy. Working in bladder cancer, we once again sought to improve this checkpoint therapy by using functional genomics and synthetic lethal concepts.

Therefore, we used a shRNA silencing library that was inserted into murine bladder cancer cells; these cells were then injected in animals, leading to tumors. These tumors were randomly assigned to be either treated with checkpoint inhibitors or carrier solution. After treatment, the tumors were removed and sequenced and then we examined which genes represented by depletion constructs had been lost in the treatment arm but still present in the control arm. This led us to a stunning discovery that the loss of the collagen receptor DDR2 and other genes such as those involved with CCL2/CCR2 leads to significant loss of cancer cell presence.

Individual examination of DDR2 and CCL2/CCR2 confirmed results from the library approach. The reason why both DDR2 and CCR2 were attractive targets was because both had small molecule inhibitors and therefore we are able to show proof of principle of therapeutic combinations of checkpoint inhibitors with small molecules that lead to significant enhancement of checkpoint inhibition therapy period importantly this work was shown to be relevant not only in bladder cancer but across several other major human cancers such as colon cancer breast cancer

and Melanoma.[3,14] We are very excited about these findings and are in current discussions with several pharmaceutical companies to extend these results in the clinical trial setting.

Q5. On Co-expression Extrapolation (COXEN): Genes for predicting the response of tumor cells to a specific drug treatment (see also, NCT02177695, https://clinicaltrials.gov/ct2/show/NCT02177695)

A. The primary focus of this study was to use a Computer algorithm program called coexpression extrapolation or "COXEN" to identify tumor biomarkers that may predict a patient's response to chemotherapy before surgery. What made this computer algorithm unique was the fact that it allowed extrapolation of drug sensitivity data from cell lines grown in a culture dish onto the development of predictive markers of response in human cancer. Developed in 2007, this idea was a conceptual breakthrough at that time.[15,16]

In this randomized controlled trial, the COXEN program did not select a patient's therapy; rather the chemotherapy that he/she received will be either Arm 1 (gemcitabine and cisplatin) or Arm 2 [methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim)]. A cohort of 167 evaluable patients took part in this study. The patient's response to chemotherapy tests the usefulness of biomarkers generated by the COXEN program to predict response. Results from this trial revealed that treatment-specific COXEN scores were not significantly predictive for response to individual chemotherapy treatment. This was not very surprising since the most active agent in both the chemotherapeutic regimens was cisplatin and therefore it would have been highly unlikely that given the design of the algorithm that the prediction could have stratified between these two regimens.

However, we are very encouraged to see that COXEN score was significantly associated with downstaging in the pooled arms of the trial which aggregated all patients. Therefore it suggests that COXEN predicts the outcome of cisplatin based chemotherapy but further examination and trials need to be done. Most importantly however it demonstrates in a prospective clinical trial that an algorithm that uses drug sensitivity information from cell culture can effectively develop gene expression biomarkers that predict outcome to some degree in patients which is a major conceptual advance scientifically.

Dr Theodorescu also recently developed the Molecular Twin initiative. For further reading on this topic, please see:

1. Molecular Twin initiative – https://www.cedars-sinai.org/newsroom/molecular-twin-initiative-will-help-advance-precision-cancer-treatment/
2. Virtual Cancer Center – https://www.cedars-sinai.org/newsroom/cedars-sinai-and-usc-to-jump-start-young-scientists-seeking-to-cure-cancer/
3. Major Discoveries See also https://www.cedars-sinai.edu/research/labs/theodorescu/areas.html. JoPM

Dr Dan Theodorescu

Dr. Theodorescu is internationally known for his work on the molecular mechanisms driving bladder cancer and tools that determine drug response as well as discovery of new drugs for bladder and other cancer types. Examples include discovery of genes that regulate tumor growth and metastasis and novel biomarkers and concepts for precision therapeutic approaches such as the COXEN principle tested in a national (SWOG 1314) clinical trial.[1] He also conceptualized the approach and then led the discovery and development of a "first in class" RalGTPase inhibitor as a new therapeutic in cancer.[2] This drug was awarded a US patent and is in commercial development. Recently he identified approaches that define effective combination immunotherapy with checkpoint inhibitors[3] and a new bladder cancer subtype, called "C3" whose presence in tumors predicts immunotherapy response.[4]

He has also discovered mutation specific pathways regulating expression of TERT TElomerase Reverse Transcriptase (TERT) a rate-limiting step in reconstituting telomerase activity which maintains telomere length and cellular immortality, a cancer hallmark.[5,6] Throughout his career he has written over 350 articles and reviews on bladder cancer and precision medicine.[7-13] Theodorescu is a founding co-editor in chief of *Bladder Cancer*, the first journal focused on this disease and an elected member of the American Society for Clinical Investigation (ASCI), Association of American Physicians (AAP), the American Association of Genitourinary Surgeons (AAGUS), the American Surgical Association (ASA) and the National Academy of Medicine (NAM). He is an Honorary Fellow of the American Association for the Advancement of Science (AAAS).

- Discoverer of the metastasis suppressor, RhoGDI2, using a novel approach that combined gene expression profiling of cell line data with that of clinical tumors. This work was among the first to demonstrate the utility of this approach in finding both mechanistically and clinically relevant metastasis regulators.
- Developer of a means of extrapolating therapeutic research on cell lines, to effectively predict therapeutic response in human tumors. This novel approach led to the development of COXEN (CO-eXpression ExtrapolatioN), a radical strategy based solely on in vitro assays and aimed at personalizing cancer therapy and identifying which new drugs have a high likelihood of being effective in patients.
- Discoverer that Ral GTPase as a downstream effector of Ras is a major driver of bladder cancer through a novel mechanism involving CD24 which is also in turn regulated induced by the androgen receptor. This discovery demonstrated a novel mechanism by which the androgen receptor mediates bladder cancer growth and progression. Since there are U.S. Food and Drug Administration–approved therapies to target the androgen receptor, this work has also established the notion of treating bladder tumors with high CD24 expression using androgen receptor blockers.

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