From the laboratory to the clinic

PROFESSOR JEFFREY TORETSKY

Professor Jeffrey Toretsky is a medical doctor with a passion for scientific discovery that will benefit patients. In this elucidating interview, he describes his development of a targeted treatment for a rare cancer, the translatability of his research and the importance of moving between disciplines

Why have you chosen to focus on Ewing Sarcoma (ES) in your work developing targeted anticancer therapies? What is the prognosis for sufferers of ES and could you outline the therapeutic options currently available to them?

Cancer patients, overall, need better therapies. Many ES patients will survive with current therapy, but I cannot accept 30-40 per cent of patients dying (and neither can their loved ones). All patients receive a cocktail of five drugs, given in alternating combinations of two (ifosfamide and etoposide) and three (vincristine, doxorubicin and cyclophosphamide). They also require local tumour management, such as radiation or surgery. Those who survive suffer a high incidence of late effects – cardiac, neurologic, orthopaedic and others.

We developed a programme to target EWS-FLI1, a fusion protein unique to ES, which could directly benefit ES patients and indirectly benefit many other patients through paradigms for treating other cancers.

EWS-FLI1 is critical to the oncogenesis and maintenance of ES. Could you explain the effect of YK-4-279 on this protein and how it inhibits the growth of ES tumours?

EWS-FLI1 can cause non-cancer cells to develop cancer-like properties, a process called transformation. Our work shows YK-4-279 directly affects three aspects of EWS-FLI1 function: it disrupts the binding of RNA Helicase A, a key functional partner; reduces transcript activation; and alters EWS-FLI1 alternate polyadenylation of cyclin D, preventing formation of a more oncogenic isoform. Through these three effects, and additional mechanisms under investigation, YK-4-279 causes apoptosis of ES cells.

The US Food and Drug Administration (FDA) has given YK-4-279 orphan drug status under the name Efdispro® and it has been tested on a rat model of ES. What is required to bring the drug to human clinical trials?

The rat model allowed us to deliver continuous dosing of YK-4-279 over roughly one month, which led to regression. This was the first time ES was modelled in a rat; the paper was published in *Oncotarget* in November 2013.

The biggest remaining hurdle to a human clinical trial is developing a usable formulation. This is currently the top priority of the company that is developing YK-4-279.

How translatable is this research to other cancers?

We have evidence that other cancers respond to YK-4-279, as we published in 2012. Very few small molecules have exactly one biologic target. We are currently working to understand the interactions of YK-4-279 with other cellular proteins, in particular, those closely related to FLI1.

Whenever you identify a target for cancer therapy, you have to find what's unique about it, making it important for cancer and not normal cells. We believe some transcription factors interact with partner proteins differently in cancer cells. EWS-FLI1 is a great example of that, as a transcription

Targeted cancer therapy

Researchers from **Georgetown University Medical Center** have uncovered a novel treatment for Ewing sarcoma. Investigators from the Toretsky Laboratory not only hope to improve survival rates for patients with this rare cancer, but their approach has the potential to create an entirely new class of cancer drugs

EWING SARCOMA (ES) is a rare cancer of bone or soft tissue. It usually develops in puberty when bones are growing fastest and typically starts in the long bones of the arms and legs, the pelvis or the chest. Few existing treatments are able to cure patients when the cancer progresses, following initial treatment, and those who do survive suffer latent and damaging effects from the treatment.

At the core of all cancer therapies is the aim to find aspects of cancer cells that are different from healthy cells; in many ways ES is an ideal disease for such therapy. The exchange of genes between chromosomes (chromosomal translocation) can cause cells to grow uncontrollably, or become cancerous. In ES, this process leads to creation of the EWS-FLI1 fusion protein. This protein is both critical to the survival of the tumour and entirely unique to the tumour cells – making it a perfect target for directed anti-cancer therapy.

However, EWS-FLI1 is a transcription factor. Transcription factors – proteins that control which genes are turned on and off – have long been considered 'undruggable'. Their lack of enzymatic domain (the typical sites used to inhibit a protein) and intrinsic disorder make their crystallisation and targeting a challenge. So, although EWS-FLI1 has long been recognised as an ideal therapeutic target, there is currently no drug in clinical use that directly targets this key oncoprotein.

Dr Jeffrey Toretsky, Professor at Georgetown University Medical Center, has overcome these biochemical challenges to develop a small molecule that inhibits EWS-FLI1, called YK-4-279, and is due to enter human clinical trials in early 2015. Furthermore, his platform to identify small molecules that interfere with transcription factor binding could extend to other cancers.

PATHWAY TO PROGRESS

YK-4-279 was first disclosed in *Nature Medicine* in 2009. Since that time, Toretsky's team has published four additional peer-reviewed papers supporting the pre-clinical development of the compound.

The road to clinical development began when Toretsky found that YK-4-279 binds to EWS-FLI1 and prevents it from interacting with RNA

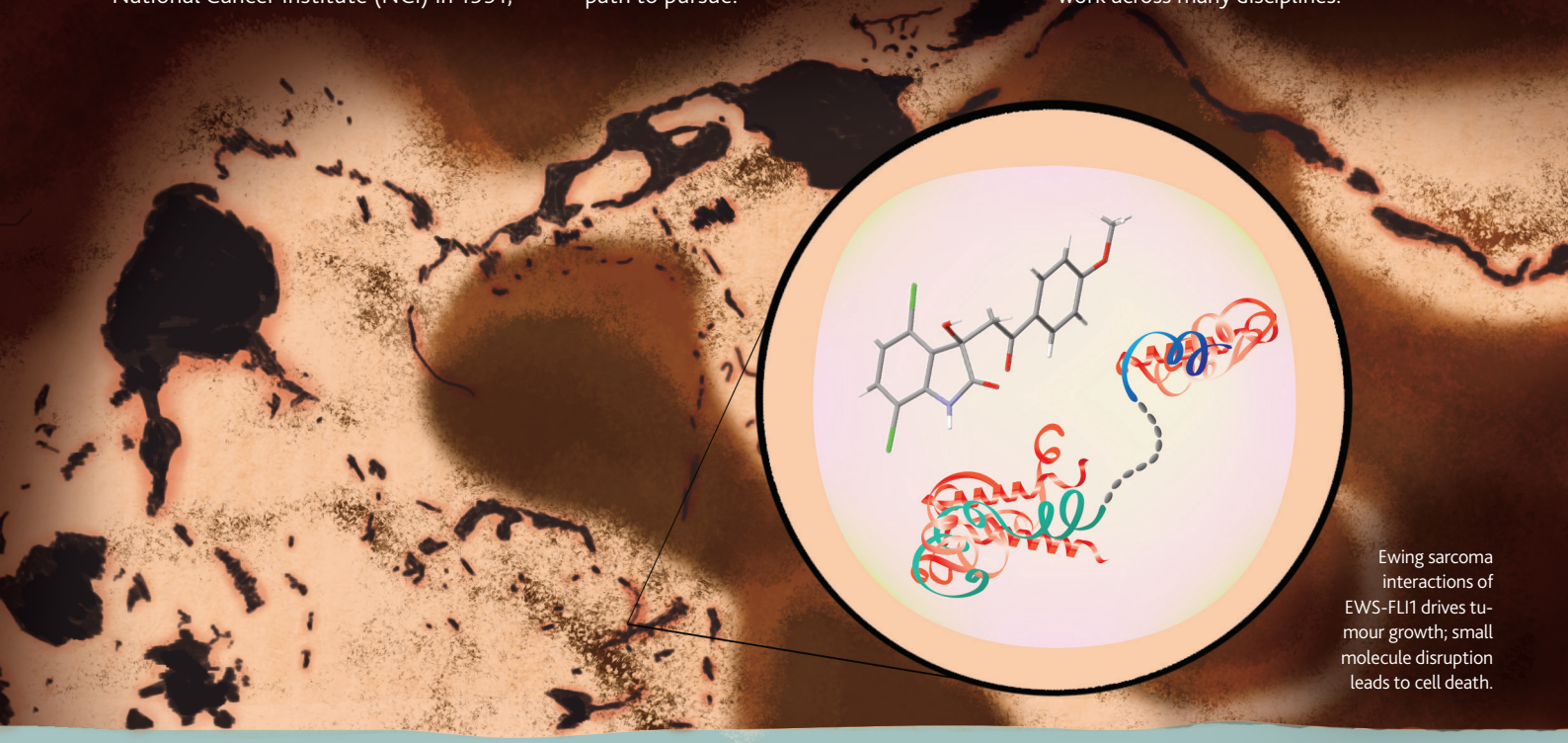
factor that doesn't even exist in a normal cell. The new approach is to take what we know about EWS-FLI1 and apply the knowledge to other transcription factors. I believe scientists will find targetable interactions between transcription factor partner proteins that are unique to malignancy; thus identifying new vulnerable aspects of cancer. This strategy is very different from most cancer research today.

What attracted you to this field of research and what continues to draw your attention?

When I started my fellowship at the National Cancer Institute (NCI) in 1991, there was a legacy of the NCI taking care of high risk metastatic sarcoma patients. In 1992, when I was choosing a topic for my research, the EWS-FLI1 fusion protein was discovered based upon the genes that are part of the ES reciprocal chromosomal translocation. For me, that discovery opened up an approach for a disease, that I recognised from personal experience, would really benefit from improved therapeutics. My goal then became targeting that unique aspect of ES. The combination of recognised clinical need and an interesting opportunity to study the biology of the disease was an intriguing path to pursue.

How important is a multidisciplinary team to your investigations?

It took a multidisciplinary team to find and optimise the small molecule lead compound. I am now part of the Biophysics Society and a member of the Intrinsically Disordered Protein (IDP) Subsection, but I'm also a paediatric oncologist. I manage patients with cancer, including ES. I've stretched myself across the scientific spectrum from learning about protein biochemistry to discovering anti-cancer targets and making new drugs. This could not have happened without collaborators to inform and support my work across many disciplines.



Ewing sarcoma interactions of EWS-FLI1 drives tumour growth; small molecule disruption leads to cell death.

Helicase A (RHA). This interaction is critical for ES cancer progression, and blocking EWS-FLI1 leads to reduced ES cell growth and apoptosis (cell death). Once the researchers had established that YK-4-279 effectively blocks EWS-FLI1, they set out to resolve the challenges of preclinical development. Successive iterations between *in vitro* studies and pharmacokinetics in two species, mice and rats, were used to explore a range of dosage regimens. Their investigations revealed that cancer cells require continuous exposure to the drug for maximum results, leading Toretsky to select a continuous infusion model for *in vivo* efficacy testing.

Continuous infusion of YK-4-279 in a rat model not only stopped progression of ES, 30 per cent of the tumours actually regressed. Some of the tumours even regressed to a point where cancer cells could no longer be detected by microscopy. Pharmacokinetic models were validated in an ES xenograft mouse (the tumour tissue was derived from another species), which also showed tumour regression, molecular targeting and apoptosis. Further, Toretsky published NCI-funded research describing the compound's pharmacology and lack of toxic effects.

A major challenge in ES is that tumours often completely regress following initial chemotherapy, only to recur once the treatment ends, suggesting that an ES stem cell survives the chemotherapy. YK-4-279 goes beyond the ability of existing chemotherapy drugs in inhibiting the ES stem cell, making it an ideal candidate for a more effective and less toxic alternative.

CLINICAL TRIALS

Since Toretsky recognised the potential of ES-specific targeting over a decade ago, he has developed the first ever small molecule to directly target the causative oncogene of ES. The compound has recently been given orphan drug status by the US Food and Drug Administration (FDA) under the name Efdispro® (EWS-FLI1 Disrupting Protein). Over the next year, Toretsky will advance Efdispro® into first-in-human, first-in-class clinical trials through a company that he co-founded.

To move YK-4-279 into the clinic, further studies are required to optimise the drug. The researchers are currently searching for a suitable pharmacodynamic biomarker, a molecule or physiologic change, that can be correlated to therapeutic effect. Such a biomarker would enable identification of patients who are responding to the drug using a simple blood test. It could also be used to give optimal doses and therefore minimise toxicity.

At present, Toretsky is investigating microRNA (miRNA) – small pieces of RNA important for the biology of tumour cells that can be detected in blood. In a pilot experiment, the group was able to distinguish animals with ES xenografts from those without tumours based on their blood miRNA profiles. Going forward, the team aims to perform a series of validating experiments to determine which miRNA(s) should be measured to reflect changes in tumour growth.

In the longer term, Toretsky's team will continue to work to obtain a better understanding of how the drug interacts with EWS-FLI1. Whilst they know that YK-4-279 strongly activates apoptosis in ES cells, they do not fully understand the protein-protein interactions responsible for this rapid cell death. As part of these ongoing investigations, Toretsky has made observations that suggest YK-4-279 is also toxic

INTELLIGENCE

YK-4-279 SPECIFICALLY TARGETS ETS FAMILY FUSION-PROTEIN CANCERS IN CLINICAL TRIAL

OBJECTIVES

- To optimise delivery methods and obtain toxicologic data for submission of an investigational new drug application leading to a first-in-class, first-in-human clinical trial
- To conduct a clinical trial that would sustain future phase II clinical trials to determine efficacy of YK-4-279 as a novel anti-cancer drug in specific diseases

KEY COLLABORATORS

Aykut Üren (surface plasmon resonance); **Milton Brown** (medicinal chemistry); **Steve Metallo** (chemistry of proteins), Georgetown University • **Peter Houghton** (pharmacology/animal models), Ohio State University • **David Loeb** (cancer stem cells), Johns Hopkins University • **Phil Monroe** (pharmacology); **S Peter Hong** (pharmacology), Battelle Memorial Institute

PARTNERS

Intrinsically Disordered Protein/structural biologists • **Vladamir Uversky**, University of South Florida • **Peter Wright**, Scripps Institute • **Vladek Minor**, University of Virginia • **Maks Chruz**, University of South Carolina • **Art Palmer**, Columbia University • **Tara Snyder**, chemist, Albany Medical Research

FUNDING

National Institutes of Health (NIH) • Children's Cancer Foundation of Baltimore, Maryland • Many other philanthropic and advocate organisations

CONTACT

Professor Jeffrey Toretsky
Principal Investigator

Departments of Oncology and Pediatrics
Lombardi Comprehensive Cancer Center
Georgetown University
3800 Reservoir Rd, Washington, DC 20057, USA

T +1 202 687 8655
E jat42@georgetown.edu

http://projectreporter.nih.gov/project_info_description.cfm?aid=8047311&icde=19005995&ddparam=&ddvalue=&ddsub=&cr=3&csb=default&cs=ASC

JEFFREY TORETSKY was recruited to Georgetown University where he was granted tenure in 2005 and achieved the rank of professor in 2011. He was inducted into the American Society of Clinical Investigation in 2007 and received the Burroughs-Wellcome Clinical Scientist Award in Translational Research in 2008. Toretsky's lab is currently focused on investigating the effect of EWS-FLI1 upon RHA as a helicase.

to other cancers, including prostate carcinoma. As the drug heads for clinical trial, the potential that it could be used to treat other cancers is being considered.

EXTENDING TARGETS

Whilst the key focus of Toretsky's work is directly targeting EWS-FLI1, he also aims to characterise the biochemical nature of the protein, which exhibits properties associated with an intrinsically disordered protein (IDP). A resolved 3D understanding of EWS-FLI1 will not only enhance YK-4-279 optimisation, but may have a far reaching impact, as IDPs are recognised as crucial players in many diseases.

Taking an unconventional approach, Toretsky has met the challenge of targeting 'undruggable' transcription factors. By combining the power of laboratory studies, modelling and pharmacokinetics, he has identified, optimised and progressed a novel, targeted small molecule that could be used as an archetype for cancer treatment. Toretsky believes it could go even further: "If I can get cancer researchers to engage colleagues who are doing brilliant work in protein chemistry and biophysics, and apply knowledge that medical research has shown over many years regarding pharmacology and small molecules, I think there will be a whole new way of looking for and identifying potential targets for many diseases, not just cancer".

The route to clinical trial

1992: Toretsky begins studying Ewing Sarcoma (ES). His early work involves investigations of minimal residual disease in ES patients

2006: The Toretsky lab identifies RNA Helicase A (RHA) as a partner of EWS-FLI1

2009: EWS-FLI1 is identified as a target for YK-4-279. This small molecule is shown to prevent RHA from interacting with EWS-FLI1 and slow the growth of ES xenografts, in pivotal research published in *Nature Medicine*

2010: Further National Cancer Institute (NCI)-funded research describes the pharmacology and lack of toxicity of YK-4-279 and shows the superior ability of the compound to inhibit the ES stem cell

2011: Toretsky establishes TDP Biotherapeutics, Inc., obtaining exclusive license for the commercial development of YK-4-279

2012: The United States Patent and Trademark Office (USPTO) issues the patent for YK-4-279 to Georgetown University

2013: The team creates the first rat xenograft model of ES and treats it with YK-4-279. The research, published in *Oncotarget*, makes several key findings:

The pharmacokinetic properties of YK-4-279 suggest a need for the continuous infusion of the compound

The compound induces cell death and reduces EWS-FLI1 regulated caveolin-1 protein

In vivo administration reduces xenograft growth; a sustained complete response is seen in over 30 per cent of ES tumours

These results provide the pre-clinical evidence needed for YK-4-279 to progress to clinical trial

2013: YK-4-279 is given orphan drug status by the US Food and Drug Administration (FDA) under the name Efdispro®. Formulation for human use and toxicology is underway prior to an FDA investigational new drug (IND) application

2015: The Efdispro® clinical trial is due to begin

EWING SARCOMA

- Affects **3** people per million every year
- Around **500** people are diagnosed in the US every year
- Largely affects people between the ages of **10** and **35**
- Local disease: five-year survival rate of **73%**
- Metastatic disease: two to three year survival rate of **20-30%**
- Metastasis at the time of diagnosis is found in around **1/3** of children
- Most common in pelvis, femur, humerus, ribs and clavicle
- Symptoms include fever, anaemia, swelling and bone pain
- **85** per cent of ES cases are the result of a translocation between chromosomes **11** and **22** – fusing the EWS and FLI1 genes to create the EWS-FLI1 fusion protein