mAb attack on WT1

By Kai-Jye Lou, Staff Writer

Wilms tumor 1 is overexpressed in many different cancers, but most antibody and small molecule developers have deemed it undruggable because it is an intracellular transcription factor. Now, researchers at **Eureka Therapeutics Inc.** and the **Memorial Sloan-Kettering Cancer Center** have targeted Wilms tumor 1-positive cancer cells with a mAb against peptide fragments derived from the protein that are presented on the cells' surface.¹

The biotech hopes to have the mAb in a Phase I trial in leukemia within two years and is seeking a clinical development partner.

Wilms tumor 1 (WT1) is a transcription factor found in the cell nucleus that is overexpressed in a range of leukemias and solid tumors.

Ongoing efforts to address WT1-positive cancers have focused on vaccines and T cell therapies as opposed to antibodies and small molecules.

Antibodies are unable to target intracellular proteins, whereas small molecules typically cannot modulate the protein-protein interactions in which transcription factors are involved.

In 2006, research teams led by David Scheinberg, chair of the Molecular Pharmacology & Chemistry Program at the **Sloan-Kettering Institute** at MSKCC, published a pair of studies identifying a series of nine-amino-acid, WT1-derived peptides that could be used to trigger a T cell immune response against some WT1-positive cancer cells.^{2,3} The peptides are processed and presented on the cell surface in a complex with a specific human leukocyte antigen (HLA) molecule called HLA-A 0201.

Like companies and other academics, Scheinberg's initial strategy was to develop a cancer vaccine because many such peptide-HLA complexes are recognized by T cell receptors (TCRs). In a pilot study led by MSKCC, the group's polyvalent WT1 peptide vaccine candidate stimulated a WT1-specific T cell response in patients with acute myelogenous leukemia (AML) who had finished chemotherapy and were in complete remission.⁴

At least three companies have clinical-stage immunotherapies for WT1-positive cancers. **Inovio Pharmaceuticals Inc.**'s WT1-targeting DNA vaccine is in Phase II testing in AML and chronic myelogenous leukemia (CML). **GlaxoSmithKline plc**'s GSK2130579A, an antigen-specific cancer immunotherapeutic using recombinant WT1, is in Phase I testing in AML. **Formula Pharmaceuticals Inc.** has exclusively licensed the WT1 peptide vaccine developed by Scheinberg's group and designated it FPI-01. Scheinberg said the vaccine is being evaluated in multiple MSKCC-sponsored Phase II trials in AML, acute lymphoblastic leukemia (ALL) and mesothelioma.

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**—David Scheinberg,
Memorial Sloan-Kettering
Cancer Center**

All of the immunotherapies are being tested in patients who are in remission or have minimal disease burden rather than those with more active disease.

Because the WT1 peptide-HLA complexes are found on the cell surface, Scheinberg wanted to revisit the idea of using therapeutic mAbs to target WT1-positive cells.

"Vaccine approaches are most likely to be applicable in patients who have little detectable leukemia or cancer left," he told *SciBX*. "Antibodies can be used when there is active cancer and are also far easier to apply than a patient-specific live T cell therapy."

The MSKCC group thus teamed up with human antibody discovery and engineering company Eureka in 2010.

The research group used the biotech's phage display platform to screen for single-chain variable fragments that bound the WT1-derived peptide-HLA complexes identified in the 2006 studies. The lead fragments were then engineered into full-length human mAbs.

In panels of human cells isolated from healthy donors and patients, one of the leads, dubbed ESK1, bound to the majority of leukemic and solid tumor cell lines that were positive for both WT1 and the HLA-A0201 molecule but not to lines that were negative for one or both

markers. In a series of *in vitro* cytotoxicity studies, the mAb killed WT1-positive cancer cells via antibody-dependent, cell-mediated cytotoxicity.

In mouse xenograft models for human ALL and Philadelphia chromosome-positive ALL, ESK1 decreased tumor burden and increased survival compared with a control IgG. Some of the mice showed no evidence of disease after treatment.

Results were published in *Science Translational Medicine*.

"Our therapeutic antibody does not need to enter the cell because even though WT1 is intracellular, peptides derived from the transcription factor do move to the cell surface, where they can be targeted by our antibody," said Cheng Liu, a coauthor on the paper and president, CEO and founder of Eureka. "ESK1 works by mobilizing the host immune system to kill WT1-positive cancer cells."

The case for mAbs

Although companies and academics have advanced other immunotherapy candidates to treat WT1-positive cancers as far as Phase II trials, Liu and Scheinberg both think a therapeutic mAb will have utility because it offers the potential to treat patients with active disease.

Liu told *SciBX* that even though WT1 is overexpressed in many solid cancers, the plan is to start in hematological cancers because the endpoints are easier to monitor.

According to Scheinberg, the initial populations being considered for ESK1 are patients who have acute or chronic leukemias, myelodysplastic syndrome (MDS) or multiple myeloma (MM) who have not fared well on other treatments.

Haruo Sugiyama, a professor in the Department of Functional Diagnostic Science at the **Osaka University Graduate School of**

Medicine, said that “for cure-oriented therapy in leukemia, cancer stem cells will have to be killed.” He noted that ESK1 could have potential for eradicating leukemia stem cells if the binding of ESK1 to the WT1-HLA complex on such cells is similar enough to that of WT1-specific cytotoxic T lymphocytes, which are induced by vaccine-based strategies.

Sugiyama added that one may also want to consider combining ESK1 with an existing WT1 vaccine as this could yield better outcomes than either treatment alone. “Simultaneous combination therapy of ESK1 and a WT1 vaccine, or the use of ESK1 together with chemotherapy for remission induction and a WT1 vaccine for remission maintenance, may be advantageous,” he told *SciBX*.

Sugiyama and colleagues at the Osaka University Graduate School of Medicine are developing a WT1 peptide vaccine, which the group has in Phase II testing for various cancers.

Scheinberg said the group's next steps are to evaluate ESK1 in additional animal safety studies and to scale up manufacturing of the antibody.

“We are hoping to have our antibody reach clinical-stage testing in one to two years,” added Liu. “We would like to engage with an industry partner that has extensive clinical and product development experience to help with the clinical development program.”

MSKCC and Eureka have an ongoing discovery and development collaboration that provides both parties with co-ownership rights to

resulting drug candidates and related IP. The partners have cofiled for patents covering ESK1. The technology is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

Eureka Therapeutics Inc., Emeryville, Calif.
Formula Pharmaceuticals Inc., Berwyn, Pa.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
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