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To The Next Level

Dr. Nabil Ahmed and Dr. Stephen Gottschalk

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Cancer researchers Dr. Nabil Ahmed and Dr. Stephen Gottschalk are tweaking immunologic technology to attack osteosarcoma, a cancer that can hide from the usual immune cell strategies.

A NASTY CANCER

Osteosarcoma, although not a very common malignancy, is the most common human primary bone cancer, accounting for over 900 new cases in the U.S. and about 200 cases in the U.K. each year. Osteosarcoma characteristically attacks the long bones, like the legs and arms. More importantly and more distressing, osteosarcoma is most prevalent in children and younger adults less than 25 years of age. Current therapeutic strategies usually consist of radical surgery, often an amputation, and systemic chemotherapy. However, despite improvements in outcome for patients with local disease, say, isolated in the leg bone, the survival rates for patients with metastatic or recurrent disease remain very poor. Given these facts, scientists like Nabil Ahmed and Stephen Gottschalk have been researching novel biologically-based and targeted therapies using clever immunologic manipulations aimed at a protein called HER2.

Some cancer types, most famously breast cancer, can express HER2, or human epidermal growth factor receptor 2. HER2 is so named because it has a similar structure to normal protein human epidermal growth factor receptor—HER1. HER2 production, like the production of HER1 and other forms of epidermal growth factor receptor, can promote cell growth and division when it is functioning normally. Indeed, these proteins are crucial for the in utero development of the heart and major vessels. On the other hand, when HER2 is overexpressed after birth, cell growth accelerates beyond its normal limits and in some types of cancer it can promote rapid cell growth and proliferation—hence tumour formation. It is well known that HER2-positive breast cancer tumours are more invasive and aggressive than HER2-negative tumours. HER2-positive tumours are associated with increased disease recurrence and poor prognosis. Over-expression of HER2 is also associated with more aggressive types of ovarian, stomach, and uterine cancers. Thus, HER2 has been targeted in recent years with a variety of immunological cancer drugs.

For example, trastuzumab (sold under the brand names Herclon and Herceptin) is a monoclonal antibody that binds to and interferes with the HER2 receptor. This antibody, combined with other chemotherapeutic drugs, has been show to improve overall survival and disease-free survival relative to treatment regimens involving treatment with placebo or chemotherapy alone. But to work, trastuzumab must bind to the receptor—HER2—on the outside of the tumour cell, preventing it from being activated and "doing its thing." HER2-negative tumours and tumours with low expression of HER2 are not vulnerable to trastuzumab and similar monoclonal antibody drugs. This is precisely the problem with osteosarcoma. Although HER2 is expressed in over 60% of primary osteosarcoma tumours and correlates with a poor outcome, its expression on osteosarcoma cells is low, rendering HER2-specific antibodies like trastuzumab less effective. This low profile condition in osteosarcoma is what Ahmed, Gottschalk and their colleagues are attempting to circumvent.

CHIMERISM: RESURRECTING A MYTHOLOGICAL HYBRID

In order to kill a cancer cell, the tumour cell must exhibit certain antigens on its surface that can be processed to activate cytotoxic T cells, those T lymphocytes that attack cancer cells and cells that are infected by viruses. However, cancers like osteosarcoma do not attract and allow activation of cytotoxic T cells, thus evading the usual anti-tumour immune process. This is where Ahmed and Gottschalk plan to use a hybrid—a chimera—to immunologically attack osteosarcoma cells. The hybrid in question is a chimeric antigen receptor, or CAR.

The chimera, a creature from Greek mythology, is an animal with a body made up of parts of more than one animal. Homer's Iliad describes "a thing of immortal make, not human, lion-fronted and snake behind, a goat in the middle, and snorting out the breath of the terrible flame of bright fire." Ahmed and Gottschalk's chimera, on the other hand, is not so fearful, but it is just as fantastic. The CAR that Ahmed and Gottschalk wish to use to treat osteosarcoma is a molecule that seems to be made of different parts—one side consisting of the extracellular receptor domain of a monoclonal antibody and the other a so-called transmembrane and cytoplasmic signalling domain derived from molecules that are known to activate T cells so they become cytotoxic. In other words, one side of the CAR is an antibody to bind to HER2 receptors in the tumour—the head of a snake, able to bite and hold on to the tumour—and the other side is a T cell activating molecule—the head of a lion, able to roar out an invitation calling T cells to join the party. Even more, T cells isolated from the patient's own blood cells can be grafted with this hybrid molecule, making it that much more of a hybrid, but insuring the T cell is close by when the antibody portion attaches to the tumour cell. This is the HER2-CAR T cell, designed to attach to the HER2 receptor and kill cancer cells. This amazing immunologic chimera, then, is not something for Bellerophon to kill—it is the basis for Ahmed and Gottschalk's strategy to defeat osteosarcoma.

STARTING SMALL: HOW ABOUT MICE?

Talking about CAR T cell therapy—unleashing the chimera on cancers—is fine and good, but what about data? Ahmed and Gottschalk are not simply putting down ideas on paper; they have experience—first in mice. Ahmed, Gottschalk and their colleagues developed a lung metastatic model of osteosarcoma in mice, with very promising results. They demonstrated in mice that various subtypes of osteosarcoma express HER2 at levels that, in contrast to HER2 antibodies like trastuzumab, can be efficiently targeted using CAR T cells decorated with the HER2 targeting CAR. Indeed, the investigators found that there is a higher level of HER2 expression on osteosarcoma stem cells makes them particularly susceptible to HER2-CAR T cells despite being generally resistant to chemotherapy typically used in osteosarcoma regimens. In the mouse model, HER2-CAR T cells exhibited a potent anti-tumour effect even against bulky, established lung and other vascularized osteosarcoma metastases. They published their data in the journal Molecular Therapy in 2009, and numerous researchers since then have referred to it. Ahmed and Gottschalk used this data as the justification for launching a first-in-man trial using autologous HER2-CAR T cells for advanced osteosarcoma.

THE CHIMERA IS SAFE AND EFFECTIVE

With the efficacy of HER2-CAR T cells in mice established, Ahmed and Gottschalk turned to human disease. They and their colleagues took patients with recurrent or refractory HER2-positive sarcoma received escalating doses of HER2-CAR T cells to assess effectiveness and safety of the approach. After all, the chimera may attack the cancer—which is a good thing—but it may also attack the patient's own cells. In a study named ***HEROS,*** that is HER2 for OSteosarcoma, that was later published in the May 2015 issues of the Journal of Clinical Oncology, they took 19 patients with sarcomas—16 of them with osteosarcoma—and treated them with HER2-CAR T cells. These HER2-CAR T cells were derived from T cells removed from the patients' own blood. These hybridized cells were tested from each patient and all were found to be toxic for HER2-positive target cells in vitro. After treatment, they detected HER2-CAR T cells for at least 6 weeks in the majority of patients, especially those who received higher doses of HER2-CAR T cells.

The development of effective targeted therapies for osteosarcoma is hampered by the limited number or low expression of known tumour antigens, such as HER2. HER2, one of few antigens expressed by osteosarcoma, is quite attractive for immunotherapy since it is expressed in > 60% of primary osteosarcoma and correlates with a poor outcome. But although HER2 is a validated target for breast cancer immunotherapy, its expression on osteosarcoma cells is low, rendering HER2-antibody therapy less effective. That's where the HER2-CAR T cell comes in.

The results of the study were exciting. Two of the patients actually had their tumours biopsied six and 12 weeks after the infusion and HER2-CAR T cells were detected at tumour sites. So the chimeric cells actually made it to the target. Of 17 evaluable patients in the study, four had stable disease for 12 weeks to 14 months after the treatment. Three of these patients had their tumours removed. In one, the tumour had actually undergone necrosis by almost 90%. So the chimeric cells actually did the work they were designed to do.

The median overall survival of all 19 infused patients was 10.3 months, with a range of 5.1 to 29.1 months, which was good considering these were patients who had recurrent or refractory disease and most had bulky disease at the time of HER2 CAR T cell administration. With this study, Ahmed, Gottschalk and their associates had the first evaluation of the safety and efficacy of HER2-CAR T cells in patients with sarcomas. They showed that the cells can be given systemically without evident toxicity to the patient, setting the stage for studies that combine HER2-CAR T cells with other immunomodulatory approaches to enhance their expansion and persistence and combat difficult cases of cancer, including osteosarcoma.

WHERE DO THEY GO FROM HERE?

Based on their encouraging work with HER2-CAR T cells and sarcoma, both in mice and in humans, Ahmed, Gottschalk and their associates now want to expand their scope to develop an effective immunotherapeutic approach to progressive osteosarcoma. In a study design that has already been approved by the National Institutes of Health's Recombinant DNA Advisory Committee (RAC), the Institutional Biosafety Committee of Baylor College of Medicine and by Baylor's Institutional Review Board, the researchers have initiated a study that builds on their exciting findings, entitled Administration of HER2 CAR Expressing T cells to Subjects with Advanced Osteosarcoma: NCT00902044. This study, named ***HEROS 2.0,*** Ahmed and Gottschalk will give autologous HER2 CAR T cells after creating a "lymphocyte space" that will be permissible of expansion the administered T cells and their persistence past the 6 week period seen with The HEROS Study.

With this study, Ahmed, Gottschalk and their colleagues plan to enrol patients with progressive HER2-positive osteosarcoma who have failed standard-of-care therapy. These patients will be treated with HER2-CAR T cell infusion, provided that: their T cell lines can be properly modified to work; they have a life expectancy of more than six weeks; they an appropriate physical activity scale; and acceptable organ function for the therapy. In the first human study, Ahmed and Gottschalk assessed about eight to twelve patients per year for enrolment, with approximately 80% of them being found to have HER2-positive tumours. With the same attrition factor as the first, smaller study—which was about 25%—recruitment and infusion of 12 to 15 patients would be feasible over a projected two-year study period.

One important question the researchers need to answer in The HEROS 2.0 Study is the question of lymphodepletion. When T cell therapies are used in patients—such as here, with HER2-CAR T cells being administered to fight the cancer—the native T cells in the patient's body tend to inhibit the proliferation of the administered therapeutic T cells via a normal T cell regulatory mechanism mediated by regulatory T cells, or Treg cells. Obviously, a person's T cells

cannot replicate out of control—that would be tantamount to a case of leukaemia. The body has mechanisms in place to prevent such proliferation. However, if you plan to inject someone with T cells designed to kill the cancer cells, you would like them to multiply and spread throughout the body, essentially on a search-and-destroy mission. The more soldiers in your army, the better you are at searching out the enemy. One strategy to achieve this, is to treat patients before HER2-CAR T cell infusion with the chemotherapy medications cyclophosphamide (CY) and fludarabine (FLU), drugs that kill of many of the native T cells in the body. That way, the HER2-CAR T cells are free to multiply unregulated—at least until the normal T cells can regenerate themselves--and search out cancer cells to attack. The researchers will use different regimens of CY, FLU, or both to see if lymphodepletion is truly necessary during HER2-CAR T cell therapy, and if so, how much depletion is necessary.

WHAT DO THEY HOPE TO FIND?

In the end, though, Doctors Ahmed, Gottschalk and their co-workers plan The HEROS 2.0 Study to answer three questions:

First, is it safe to use autologous HER2-CAR T cells in patients with advanced HER2-positive osteosarcoma after lymphodepleting chemotherapy? In their original study, HEROS, the researchers found no ill effects. Here, however, when you deplete the patient's own T cells so you can infuse them with HER2-CAR T cells, will that cause any problems?

Second, is lymphodepletion necessary—and how much of it—in using HER2-CAR T cell therapy against osteosarcoma? In their smaller study, they did not use lymphodepletion, but some authorities feel that it gives a better result, even though it might expose the patient to obviously toxic chemotherapy. Using several levels of CY/FLU lymphodepletion therapy should help answer that question.

Finally, how long will the HER2-CAR T cells persist in the patients' bodies, will they multiply and expand their numbers and will they attack the tumour cells successfully? In HEROS, things looked promising, but they only have data on a small number of patients. Here is the major aim of the study. After all, advanced osteosarcoma is the target and HER2-CAR T cells are the bullets. Ahmed and Gottschalk need this study to show how close they can hit the bull's eye.

Researcher Profile

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Dr. Nabil Ahmed received his MD and an MPH in Paediatrics from Cairo University Kasr El Aini School of Medicine, Cairo, Egypt He did residency training in Paediatrics at the Lincoln Hospital, Weill-Cornell Medical College, in New York City and Children's Hospital of New Jersey, Mount Sinai School of Medicine, in Newark, New Jersey. He also received extensive fellowship training in Paediatric Haematology and Oncology from the National Cancer Institute at Cairo University, as well as the Texas Children's Cancer Centre at Texas Children's Hospital, Baylor College of Medicine in Houston, Texas. Dr. Nabil Ahmed's research interests include novel immunologic and gene therapies for the treatment of solid malignancies. His preclinical efforts focus on the development of novel cell based therapeutics for these malignancies, with an emphasis on targeting the tumour profile, in a manner that is inclusive of the heterogenous tumor antigenic landscape as well as elements of the tumour microenvironment.

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