

### INTERVIEW

## The Utilization of CAR-T therapy for Ovarian Cancer



**JANOS L TANYI** graduated summa cum laude from the University of Debrecen, Hungary with a degree in medicine. He completed residency training in obstetrics and gynecology at the Baylor College of Medicine in Houston, and a gynecologic oncology fellowship at the University of Pennsylvania (UPenn). Dr Tanyi also obtained PhD degree in 2008 at the Semmelweis University, Budapest. His research identified lysophosphatidic acid as an important therapeutic target for ovarian cancer. Dr Tanyi was then recruited as an assistant professor to the Department of Obstetrics and Gynecology at UPenn. His clinical and translational research focuses primarily on tumor immunology and immunotherapy. This includes identifying mechanisms by which tumors escape immune attack and developing personalized immunotherapies like dendritic cell vaccines and CAR-T technology for ovarian cancers. Dr Tanyi has participated as a principal investigator in multiple clinical trials and laboratory investigations. He has authored over 80 scientific publications, six book chapters and given a large number of invited lectures worldwide and nationwide.

**Q** Could you provide an overview of the treatment options for ovarian cancer?

**JLT** : The first line treatment for ovarian cancer is surgery and chemotherapy, which can be approached in two different ways. In the first approach, there is the primary debulking surgery removing the uterus, tubes, ovaries, omentum and all visible cancer. It is followed by adjuvant chemotherapy, which is always a platinum-based chemotherapy. The other way is with three cycles of chemotherapy prior to the debulking surgery, and another three to six cycles of platinum-based chemotherapy after the patient heals from the surgery.

In terms of effectiveness, the response rate, either complete or partial is usually somewhere around 60–80%. These tumors are usually pretty sensitive for the first line of treatment. The problem is many of the patients who tend to have no evidence of disease after first-line treatment have a recurrence. At least 50–60% of patients have a recurrence about 10–12 months later and then the patient has to receive another treatment. Usually in the progression of the life of the disease, the cancer keeps recurring, and the time of recurrence get closer to each other. Resistance to the platinum-based chemotherapy gradually develops so the cancer is not as sensitive to this type of chemotherapy as it was at the beginning so other less effective single therapy treatments of chemotherapeutic agents are applied. Unfortunately, the 5-year survival of an advanced stage 3 or 4 disease is between 38 and 40%, that is, less than half of patients reach 5-year survival.

There has been lots of effort over the last decade to find new therapeutic approaches, but it has only changed the survival rate percentages very minimally. This is why other therapeutic approaches like immunotherapy will be a breakthrough over time in the treatment of this disease.

**Q** Given the positive clinical data for liquid tumors, what potential do you see for developing CAR-T therapies to treat solid tumors like ovarian cancer?

**JLT**: We hope that we will reach the effectiveness of the CAR-T cells that we have seen in liquid tumors in solid tumors. But there is a major difference between these B-cell lymphomas or leukemias and solid tumors, in terms of how they are treated with CAR-T cells. These liquid tumors are single cell diseases and all the tumor cells are the same and most are in or close proximity to the vascular bed. When CAR-T is in the vascular bed they can immediately meet the tumor cells and attack them. However, in solid tumors, like ovarian cancer, these tumors are invading the surrounding normal tissue, which makes it difficult for the T cells to reach the target and fight the cancer.

We are still working on how to, firstly, effectively migrate these T cells to the target cancer cell. At the moment, this is why the CAR-T cells cannot reach the effectiveness in solid tumors that we have seen in liquid tumors. Right now, in solid tumors the approach is to deliver CAR-T cells to the vascular bed intravenously, or into the peritoneal cavity or the pleural cavity. The CAR-T cell needs to migrate and find the target cell, which sometimes takes a couple of days to reach target. This is what we have observed in our previous clinical trials. Secondly, when the CAR-T cells migrate to the target,

the concentration of CAR-T cells is much lower than what we see with liquid tumors when all the T cells in the vascular bed immediately start to multiply and reach a very high concentration. We are working towards developing ways to effectively migrate T cells and find the target cells. Thirdly, there is the issue of T cell exhaustion. This means that the activated T cell that we give

“Mesothelin is overexpressed in almost all serious ovarian cancers, and is therefore the best studied target for developing CAR-T therapy.”

to the patients is very active, but after 2 or 3 weeks the activity of the T cells decrease. If it takes longer to get the T cell to the target or the T cell may find a physiologic target closer on the T cell migration route, then by the time the T cell reaches the tumor it is already exhausted and might not be as effective. All these issues need

fixing for CAR-T therapy to be a reality for solid tumors.

**Q** What are some of the different approaches that researchers are taking to generate CARs for ovarian cancer?

**JLT** : There are only a couple of trials which use CAR-T cells against ovarian cancers and almost all of them target mesothelin. Other targets include, cMet and vEGFR. But mesothelin is the best among all targets identified thus far because it's overexpressed in most of the ovarian cancer patients and is not expressed in most normal tissues. In addition, mesothelin is overexpressed in a couple of other solid tumors and therefore, it is used as a target by many investigators.

**Q** Your group is using a mesothelin-based CAR-T approach, can you share how the technology has evolved?

**JLT** : In the past, we targeted mesothelin that used an ss1 single chain variable fragment that is derived from the mouse monoclonal antibody. Now we are conducting trials using humanized mesothelin-CAR-T cells. There is a big difference between the two.

“In the future, T cells will be applied in combination with adjuvant therapies to increase the effectiveness of the therapy.”

Previously at UPenn we used electroporation to deliver CAR mRNA into T lymphocytes, and the resulting CAR was expressed on the T cell surface. But the presence and existence of a CAR on the surface of the T cell was very short lived – just

1-2 days. So, we changed the methodology to lentiviral vector transduction. We built the CAR DNA encoding the CAR, into a lentivirus vector, which cannot multiply itself. We infected the T cell with the lentivirus, to put the CAR into the T cell DNA, which makes the CAR expression permanent. We started this lentiviral vector transduction system first with an ss1 single gene variable, which we derived from a mouse monoclonal antibody, but it had a huge overlap with the human so it reacts with human Mesothelin.

With the mRNA electroporation, the T cells expressed CAR for 1-3 days, but with the lentiviral vector CAR expression continued. However, when we gave the CAR-T cells to the patient they started to migrate and expand, but they lasted only up to 28–36 days and no longer. The reason for this in our opinion was because the human body developed an anti-CAR antibody against the mouse sequence in the ss1 single chain variable fragment of the CAR-T cell.

For the new human trials, we have made another change to using humanized antibodies instead of the mouse antibodies. Now, we see that the CAR-T cells exist for far longer period in the patients' peripheral blood. They don't disappear and it's a huge difference. If you go back to the CD19 CAR-T cells against B-cell lymphomas, it has been published that the CAR-T cells exist up to 11 years after the primary infusion.

They started developing the CD19 CAR-T cells a decade ago. We are far behind with solid tumors, we have only started to conduct these experiments in the last 3.5 years. But we are collecting data and we are improving. We have improved the effectiveness of the CAR from first to second generation and now we are using third generation. The 41BB co-stimulatory domain, and other co-stimulatory domains are built into the third-generation CAR, making them much more effective than the previous generations.

**Q** How has the evolution of the technology influenced the clinical data?

**JLT**: When you give a CAR-T cell, the T cells are from the patient, but the CAR is building extra genetic material. If there is any difference,

compared to the patient genetic material, it is recognized as foreign. The body develops anti-CAR antibodies and generates a response against the foreign material. This will eradicate the CAR-T cells.

In our mouse anti-mesothelin experiments, we saw CAR-T expression up to 28–36 days in the peripheral blood of the patient. With the humanized CAR-T cells we have seen a far longer existence so far, which is very promising.

With this kind of persistence, the T cells are active and working and this can create a problem called antigen deletion. After the CAR-T cells identify and kill the cancer cells that overexpress mesothelin, these antigens start disappearing. But there are still a high number of active T cells searching for new targets. They then start finding the normal mesothelin-expressing cells like pleura, or pericardium, peritoneum, tonsils and can create off-tumor on-target toxicities.

So far with the ss1 mouse anti-mesothelin CAR-T cell we have not seen any off-tumor on-target toxicity. In patients, we have seen a nice expansion of T cells, so the concentration in the blood is increasing. We did tissue biopsies and we have seen nice migration of the T cells to the target.

The clinical effectiveness was proved by the fact that at day 28, 9 patients out of 15 patients we infused had stable disease and their disease did not progress for certain time period after the T cell disappeared. With the humanized T cells, we have started Phase 1 studies for testing the safety and feasibility, and what we see so far is the persistence of T cells and we are collecting more data at the moment.

Regarding the safety, the treatment was considered safe throughout the trial. We neither saw any major allergic reaction at the time of infusion, nor any cytokine release syndrome. Only one patient was diagnosed with compartmentalized cytokine release type syndrome, but that was a compassionate trial, not the main trial.

**Q** What's next in terms of CAR-T evolution to better suit the treatment of ovarian cancer?

**JLT** : We have to think in two directions. Firstly, is the concern on safety and feasibility. For example, what happens when these T cells start attacking normal cells because of antigen deletion or because they encounter mesothelin-expressing normal cells? We have to build in a stop mechanism.

There is one way to stop these T cells, simply using cyclophosphamide, which will stop the clonal expansion. At the same time, we can build in suicide genes to the particle T cells and given a chemical, these particle

clone of T cells stop multiplying and die. This will be a future step that will be applied if we see that the T cells are dangerous without using any stop mechanism.

In the future, T cells will not be applied alone. Instead, they will be applied in combination with checkpoint inhibitors or any form of adjuvant therapy, which increases the effectiveness. So far we have used two different protocols, one using lymphocyte depletion and another without, to try and compare effectiveness, expansion and existence of T cells. But in the future, the T cells will be applied with some combinatory immunotherapy, not alone.

**Q** What is the current status of clinical trials for CAR-T to treat ovarian cancer within the field and what are your projections for the future?

**JLT**: At the moment, trials are in Phase 1, where safety and feasibility are the primary objectives. A couple of secondary objectives include developing methods to effectively administer CAR-T to reach the solid tumor. Right now, we are applying CAR-T by intravenous infusion. Our group is considering other methodologies to avoid first passing the lungs which can cause pulmonary edema in the lung, which we have seen previously with other type of CAR-T cells.

**Q** You mentioned earlier one patient with compartmentalized cytokine release syndrome? How does that differ from other patients?

**JLT**: In compartmentalized cytokine release syndrome, the patient develops characteristics of cytokine release syndrome in a compartment. In this instance, the patient developed the syndrome in her pleura cavities. She had an extremely high number of T cells present and extreme fluid production – over the days as we checked she developed 4–5 liters. She showed all the characteristics of cytokine syndrome just in the pleural cavities.

The reason I wrote a paper about it is because we have to understand it. When we give CAR-T cells to patients with a liquid tumor it spreads all over the body very quickly. The vascular bed of the patient is full of tumor cells and T-cells in liquid tumors, so the reactions can happen anywhere. What we saw was that the reaction happened in a compartment, which was

the pleural cavity. But the patient didn't have any compartment cytokine syndrome in the abdominal cavity.

Interestingly, this patient's CAR-T cells were present much longer in the pleural fluid than in the peripheral blood of the patient. The T cells were absent from the peripheral blood but were still present in the pleural fluid. How did they get there, how did they multiply there and create this cytokine release syndrome in this compartment? One possible explanation is that the patient had a lot of tumor on the pleural surface and therefore a high amount of soluble mesothelin in the pleural fluid, and it's possible that it attracted and stimulated a high number of T cells there.

**Q** Are there any other applications of these Mes-CAR-Ts?

**JLT** : Yes, absolutely. Most normal cells don't express mesothelin. There are a couple of normal cell types, like pleura, peritoneum and tonsils that express mesothelin. What we have to understand is mesothelin production in tumors is significantly higher than on the normal cells. These particular solid tumors are the epithelial type of malignant mesotheliomas, which have 100% overexpression of mesothelin. Another tumor type is lung adenocarcinoma, about 50% of them overexpress mesothelin. In pancreatic carcinoma, mesothelin is overexpressed 100% and therefore it is an excellent target for Mes-CAR-Ts. We already have preliminary data from the trials for pancreatic carcinoma and malignant mesotheliomas.

**Q** In terms of commercializing CAR-T to treat ovarian cancer patients, what do you see as the primary barriers?

**JLT** : One of the main barriers is the cost associated with CAR-T therapies. It requires special manufacturing facilities for production, we have one here at the UPenn, but most universities don't. The second barrier is improving its effectiveness in solid tumors and we are now working on it. In the future, CAR-T therapy will change from one CAR to multiple CAR applications at the same time. Instead of just targeting one antigen, we will be targeting multiple antigens at the same time. This will be important for ovarian cancer because there are many different cell types, which means multiple different types of antigens as well as multiple locations. This is in

contrast to B-cell lymphoma, where the CD19 is a beautiful target because 100% of tumor cells have it. In ovarian cancer, 70% of cells have one antigen, 80% have a different antigen and 30% a third, so it's a mixed population. That will be the future of CAR-T in ovarian cancer.

**Q** How do you anticipate the field evolving over the next 5–10 years?

**JLT**: If I remember back when I was a medical student, I learnt that of the first six patients who were infused with Methotrexate for the treatment of choriocarcinoma in the 60s and 70s, a 100% lethal cancer back then, a couple of patients of the early trial infusion died. It was a horrible outcome of the trial and it took a decade for researchers to figure out the appropriate schedule and dose, and today we can treat most choriocarcinomas.

The same thing will happen to CAR-T cells and its application. CAR-T therapy is extremely powerful, but when it isn't given at the right time, in the appropriate way and concentration, it can be lethal. With a lot of work, we will figure out how to apply CAR-T cells, at what concentration, where to apply them, and when to apply. For example, when combining CAR-Ts with a checkpoint inhibitor, how far or how close to checkpoint inhibitors should you apply CAR-T cells, because they are extremely aggressive with checkpoint inhibitors. There are many small but extremely important questions that need to be answered and it will take time to address them.

Thinking about 5–10 years from now, a lot of questions will be answered, and we will reach effective tumor necrosis with CAR-T cells, hopefully in ovarian cancer. I think CAR-T cells will be therapeutic, not preventive, like dendritic cells, which I believe will be mostly used as preventative immunotherapy. CAR-T cells will be a treatment, and I strongly believe that it will be part of the first-line treatment therapy in a decade.

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