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CAR-T-cell therapies in pediatric solid cancers

Dr Perez: So, welcome, everybody. I am Dr Paula Perez, I'm a Young SIOPE member, and with Dr Ewijk, we are both happy to present the first joint Young SIOPE and ESO session, and I would like to acknowledge the speaker for today's session, Dr Franco Locatelli. Dr Locatelli is our renowned expert into heart malignancies, with a special expertise in cellular therapies from bone marrow transplant and CAR-T-cell therapy. He's the Director of Pediatric Hematology and Oncology Department at the Ospedale Bambino Gesù, in Rome, as well as a professor of pediatrics in La Sapienza University, and the President of the Italian Superior Health Council. So, thank you very much for accepting our invitation, and for sharing your knowledge and your time with the young oncologist community. And now, I will give you the word, Dr Locatelli.

Prof Locatelli: Many thanks for the kind introduction, and it's a great pleasure to be here today to discuss with you the issue of the potential role of Car-T-cell therapy in A solid tumor. It's really a privilege for me to have the opportunity to discuss with all of you this important translational research approach. These are my disclosures. And let me start this presentation briefly summarizing the main advantages of immunotherapy. Indeed, activated immune cells can recognize and target remote antigens present on cancer cells, and the stimulated immune system will not necessarily target only cells that rapidly divide. It's also important to know that the immunotherapies are associated with fewer or less devastating side effects than traditional therapies, and the memory cells that can be generated through the approaches of immunotherapy may prevent cancer from returning after the initial treatment. And there are now available several lines of immunotherapy, including the checkpoint inhibitors, the antigens specific antibodies in particular, the bispecific T-cell engager, oncolytic viruses and cancer vaccine. However, today I will focus my presentation on gene-modified T-cells, mainly the CAR-T-cells which are [Audio Not Clear] lymphocyte, genetically modified to be redirected against the specific target antigen. And talking a little bit more about the differential toxicity between chemotherapy and CAR-T-cells, in the short-term, the latter can induce the so-called cytokine release syndrome, usually occurring between day plus 1 and plus 10, and the severity of this complication is strictly dependent on the tumor burden at time of treatment. There are other toxicities that can occur in the patients, including the neurotoxicity and the cytopenia, which sometimes, may be prolonged over time and associated with features of Hemophagocytic lymph-histiocytosis. And all of us are aware that there are many trials open worldwide on the use of CAR-T-cell therapy, in particular, on hematological malignancies. Indeed, among the more than 1,000 clinical trials in the world, only 53 focus on patients with solid tumor. And to the best of my knowledge, in Europe there are only three trials specifically enrolling patients with solid neoplasia. If you look at the situation on the pediatric population and you evaluate this cartoon, you may immediately realize that the number of active clinical trials is even lower than that of open in the adult population, and mainly involve neuroblastoma, sarcoma, brain tumors as the main plasia possibly treated with this approach. And also, the number of target that have been utilized for the CAR-T-cell therapy approach is limited. Indeed, again, you immediately realize that the only 6 potential targets have been employed to develop CAR-T-cell approaches in solid tumor. One of the reasons why there is a limited number

of clinical trials enrolling patients with solid neoplasia is related to the fact that there are several obstacles that have to be overcome for rendering the approach really successfully. And these obstacles mainly refer to the identification of suitable target antigens, which are either selectively or preferentially expressed by the tumor cell. We have also to overcome the obstacle of the penetration of CAR-T-cells in the tumor mass. And it's important to emphasize the concept that tumor microenvironment is preempted towards other aggression potentially de-potentiating the activity of immune approaches, not targeted to the need of T-lymphocytes to survive into the hypoxic tumor environment. And please remember that you can ask questions on my presentation and send comments at any time. In particular, I like to share with you our experience on neuroblastoma, because we have activated in Italy the first clinical trial on pediatric patients with a neuroblastoma that represents the most common malignant extracranial solid tumor. And the metastatic presentation in this disease is rather frequent, being observed in more than 50% of patients. And when the patients have a metastatic neuroblastoma, or the tumor elements carried full-based molecular features, including the amplification of MYCN, the prognosis that they can be observing in the lower part of the cartoon remains fairly poor. And more importantly, we have not been able to improve the outcome of these patients over time, despite the multimodal approaches that have been implemented combining chemotherapy, surgery, autologous transplantation, radio therapy, and immunotherapy with the monoclonal antibody. For this reason, and also considering the demonstration of activity of immunotherapy with antibody targeting GD2, which is the [Audio Not Clear] expressed in the surface of the tumor element, it's not surprising that the CAR-T-cells were considered a potential strategy for trying to threat this particular neoplasia. More in detail, five different papers have been published on CAR-T-cell targeting GD2, including construct of first, second, or third generation, namely without, with one, or with two [Audio Not Clear]. And all these studies demonstrated that the CAR-T cells can expand over time, they may induce some objective clinical response. and more importantly, the safety profile of the approach was confirmed in all the trial. So, we decided to invest on neuroblastoma, designing a third generation CAR targeting GD2 in which the customary domain that we included are CD28 and 4.1bb. We also decided to include into the CAR construct the inducible caspase-9, which is a safety switch able to lead to the apoptosis of CAR-T cells in case of undue toxicity which cannot be controlled by conventional pharmacological therapies. The choice of this combination of customary domain is the result of an extensive preclinical trial in which we demonstrated that combining CD28 and 4.1BB resulted into the better efficacy of the CAR-T cells, and in particular, into the best survival of the animals infused with neuroblastoma cell lines, and then with the CAR-T cells. The clinical trial that we designed includes patients with high-risk neuroblastoma which failed frontline therapy during the phase I and II portion of the study, while in the phase II portion, we also considered to include children with high-risk neuroblastoma and at considerable risk of relapse. We designed a classical phase I and II clinical trial in which, during the phase I portion of the study, we considered an escalating-deescalating design, starting at the dose level screening with three million of CAR-T cell versus per kilogram recipient body weight, with the possibility of increasing to 6 and 10 million in case of a good tolerability or decreasing to 2 and 1 million in case of a dose-limit in toxicity. All patients were given CAR-T cells after a lymphodepleting treatment based on the combination of cyclophosphamide and fludarabine. If you look at this cartoon, you immediately realize that we didn't observe any dose-limiting toxicity, so, we were able to demonstrate that the maximum tolerated dose, or recommended dose, is 10 million per kg. Moreover, looking in detail at this table, you can appreciate that all patients had originally a diagnosis of metastatic neuroblastoma, in many cases associated with the amplification of MYC-N, and all patients but one failed several lines of treatment. Only one patient treated in the phase II portion of the trial didn't have evidence of the disease, while the disease status at time of infusion clearly demonstrated that all patients were in relapse or had a resistant disease in all children with a suitable tumor burden. After the inclusion of the CAR-T-cells, the aliments expanded over time, reaching a peak around two weeks after the CAR-T-cell infusion, which is associated with the greatest levels of CRP and carotene, and the expansion of the CAR-T cells involved both at the CD8 and the CD4 subset of elements. Moreover, and very importantly, the GD2 CAR-T cells are able to migrate into several different sites, including the bone marrow, the cerebrospinal fluid, and also, we found in a couple

of patients the CAR-T cells in the peritoneal effusion because we performed a paracentesis in these children. The expansion of the GD2 CAR-T cells correlated with the cytokine profile and with the occurrence of cytokine release syndrome. Indeed, you can observe that the cytokine peak was higher in patients developing CRS of grade 2 to 4 and the expansion of the CAR-T cells is associated with the production of the non-cytokine involved in CAR-T-cell therapy, namely interleukin-10, IL-6, and interferon gamma. Considering that we had the opportunity to take advantage of the presence of the inducible caspase-9 safety switch, we activated the switch sub-gene in two patients who developed toxicity which was not manageable through the use of corticosteroids or tocilizumab. And you can appreciate that the inclusion of the dimerizing agent able to activate the inducible caspase-9 was associated with the rapid disappearance of CAR-T cells in peripheral valve with a subsequent regrowth of the CAR-T cells not associated with any flare of that the toxicity. Again, let me remind that you can ask questions and send comments at any time during my presentation. In times of clinical efficacy, many patients responded, and in particular, the overall response rate and the 22 patients that we've accrued in the phase I and in the initial part of the phase II portion of the study is 80%, and more than 1/3 of patients obtained accounted remission or maintained non-evidence of the disease. Also, looking at the probability of both overall and event-free survival, you can appreciate that around 50% of patients are alive at 2 years, when the expected probability of being alive in this very advanced population of patients with conventional therapy is in the order of 10%. And the results, in terms of both overall and event-free survival are even better for those patients who had a limited tumor burden at time of CAR-T-cell infusion. Let me also share with you some images, in particular, this MIGB-scintigraphy in a child with metastatic lesions, and you can appreciate that already at week six after CAR T-cell infusion, there was a complete clearance of the metastatic lesions as demonstrated by the MIGB-scintigraphy. And considering that GD2 can be expressed also on the surface of tumor elements in Ewing sarcoma and osteosarcoma, we decided to amend the protocol to treat also patients with these bone disorders, malignant disorders. And you appreciate that after the CAR-T-cell infusion, there was enormous complete clearance of the disseminated lesion in the child with Ewing sarcoma, whereas in the patients with osteosarcoma the CAR-T-cell infusion was associated with a complete disappearance of the lung metastases, as well as of the tumor lesions involving the costal bones. But talking about the use of GD2-targeting CAR-T cells offers me the opportunity to mention that also rhabdomyosarcoma may express GD2, and indeed, we are conducting an experimental preclinical work demonstrating that the infusion of GD2 targeting CAR-T cells is able to clear the tumor cells in the animals that were initially treated with infusion of the rhabdomyosarcoma cell lines, then followed by the inclusion of the GD2-targeting CAR-T cells. There is another important potential application of GD2-targeting CAR-T cells in mainly brain tumors, and we developed an experimental modal in which we infused glioblastoma cells ortho-optically into the skull of the mice. And three days later, we infused intravenously the CAR GD2-T cells that were able to migrate into the brain of the animal and to kill the tumor elements. And indeed, if you look at the bioluminescence cartoon, you can nicely appreciate that the inclusion of GD2-targeting CAR-T cells was able to completely rescue the animal from the infusion of this GD2 glioblastoma cell lines. Again, a remind for asking to raise questions if you have concerning my presentation. So, these experimental data are absolutely on-line with a manuscript that was published some years ago by Crystal Mackall group, and the investigators demonstrated a potent antitumor efficacy of GD2-targeting CAR-T cells in an experimental model of H3-K27 mutated diffuse midline glioma. So, there is certainly the future opportunity to implement GD2 CAR-T cell trial also in patients with brain tumors. And talking about brain tumors, let me touch also the issue of another potential target, namely CD276, or if you prefer, B7-H3, because B7-H3 is expressed on the cells surface of many malignant cells of brain tumors, and indeed there is a clear correlation between the histological aggressiveness of several tumors and the expression of B7-H3. And in a higher expression of B7-H3, the peak there in the red curve is associated with a poorer survival in patients with either glioma or a ependymoma. And looking at the expression of B7-H3, you can appreciate that the highest expression is found in ATRT, the atypical teratoid rhabdoid tumors, and for this reason, several groups, including my own group, decided to invest on the generation of B7-H3-targeting CAR-T cells for this rare but very aggressive tumor associated with a dismal prognosis, and you can appreciate that in comparison to CAR-T-cell targeting

CD19, those directed against B7-H3 were able to obtain a complete control of ATRT. And as I mentioned before, we also decided to invest on this type of tumor, and for this reason we have generated a CAR construct targeting B7-H3. And again, also our data demonstrate the efficacy of the approach in rescuing the expansion of tumor elements in the animal model that we have developed. Let me spend the last three slides commenting about another option for treating with CAR-T-cell high-grade glioma, that we know very well are associated with a very poor prognosis in patients. In particular, I am alluding to the possibility of treating glioblastoma with chimeric antigen receptor T-cell targeting a different antigen, namely, the alpha-2 receptor of interleukin-13. In this seminal paper that was published around five years ago in the New England Journal of Medicine the investigator demonstrated that the infusion of CAR-T cells targeting the receptor alpha-2 of interleukin-3 were able, once injected into the receptor tumor cavity, to obtain a clearance of the tumor lesions. But once the patient relapsed, the investigator decided to move to the intraventricular infusion, and you can appreciate again in this cartoon that after the intraventricular infusion of the CAR-T cells, there was a very nice regression of the tumor lesions in the brain, as well as a clearance of a metastatic lesion appearing in the spinal cord. So, also, these antigens can represent an option for developing effective CAR-T-cell therapy in CMS neoplasia. And finally, talking again about the efficacy of CAR-T cells in sarcoma, it's important to mention the possibility of developing CAR-T cell targeting the epidermal growth factor receptor 2. And in this paper published some years ago in the Journal of Clinical Oncology, you can appreciate that in some patients the inclusion of HER-2 targeting CAR-T cell was associated with a nice reduction of the tumor lesion. So, in conclusion, GD2 CAR-T cell are associated with a good probability profile which can be further increased by the use of the inducible caspase-9. Once infused, the CAR-T cells expand, induce immune activation and have a wide value distribution documenting long-term persistence. These GD2 specific CAR-T cells have a strong antitumor activity that, in a proportion of patients, is maintained over time. A low disease-burden at time of CAR-T-cell infusion is associated with better prognosis, and the other neoplasia, including CNS tumor, might benefit from GD2-specific CAR-T cells. And finally, several groups are now working to try to explore CAR-T-cell targeting different antigens, including B7-H3, HER-2, and the receptor alpha-2 of interleukin-13. And I am pretty sure that in the future we will see additional data supporting the investment on CAR-T cells in solid neoplasia for replicating the outstanding results that have been obtained and the B-cells for proliferative disorders. Finally, let me thank all the people of my group who work in the program. A special mention to Dr Quintarelli, Dr Del Bufalo, Dr De Angelis, Dr Velardi and Mara Vinci for their extensive preclinical work. Thank you.

Dr Perez: Thank you very much. It's been an incredibly interesting session. I would like to ask you, I was wondering if CAR-T cell, in your experience in neuroblastoma, if it persists, the CAR-T cell, and how long the response persists in time.

Prof Locatelli: So, thank you very much for asking me this question, which allows me to further elaborate on this issue. As you noticed, the probability of a 3-year event-free survival is approaching 30%, which is, in my personal view, a good result considering how advanced was the population of patients we treated. And in terms of persistence of CAR-T-cells, we have been able to document the detection of CAR-T cells in patients' peripheral blood up to 1 year after the infusion of this genetically modified T-cells. I didn't mention during my talk about this, the right opportunity to address also this issue that the expansion of the CAR-T cells is directly correlated with the presence of the myeloid-derived suppressor cell. In particular, the higher the percentage and the absolute number of myeloid-derived suppressor cell, the lower the CAR-T cell extension and persistence over time.

Dr Perez: Thank you very much. And I have another question. Because when we use an anti-GD2 immunotherapy, we see a lot of side effects, that's supposed to be on target because of the ATL2, like pain, low-blood pressure. So, did patients have these? So, I understand that they have a cytokine release syndrome, but do they have these kinds of symptoms during time?

Prof Locatelli: So, thank you so much again for asking this important question. The CRS mainly occur in the first 2 to 3 weeks after CAR-T-cell infusion, and it correlated very closely with the CAR-T-cell expansion. It's important to share with you the information that in terms of either the patients treated for neuroblastoma have an even higher hyperpyrexia in comparison to children treated for B-cell precursor ELL. And as I mentioned during my talk, we needed to infuse the dimerizing agent in a couple of patients who develop grade 4 CRS, not easily manageable with corticosteroids and tocilizumab. For this reason, we decided to infuse the dimerizing agent, and this treatment resulted into a rapid disappearance of clinical signs and symptoms of CRS.

Dr Perez: Okay, thank you very much.

Dr van Ewijk: May I ask something on a different level? I was very much wondering, if the patient's relapse under or after CAR-T, it could be due to that, at one point, the CAR-Ts are not there anymore, but sometimes, also, tumors, like in haemato-oncology, they change in their pattern. Do you see, for example, any change in GD2 expression after giving CAR-Ts? Is it also like being CD19 negative in B-cell leukemia, for example?

Prof Locatelli: That's a terrific question and thank you for raising it. Indeed, and those patients who relapse, the emergence of GD2 negative elements was a rare event indeed. We observed it only in two patients. So, probably the more likely explanation is that the CAR-T cells lost efficacy because of some immune suppressive de-potentiating effect displayed by the tumor cells, or by the tumor microenvironment. And as I mentioned a few seconds ago, the presence of a high proportion of myeloid-derived suppressor cells predicted a poorer expansion of the CAR-T cell elements, and also, a shorter persistence over time.

Dr van Ewijk: Thank you very much for answer. May I continue for another one?

Prof Locatelli: Yes, of course.

Dr van Ewijk: It's that I was much wondering because solid tumors express different things, and also, we know there're elements which express differentiating. Would you foresee a future where you would combine certain CAR-Ts against GD2, and for example, B7RH3?

Prof Locatelli: It's a good point. Indeed, we are thinking to prepare a CAR construct allowing the simultaneous targeting of both antigens. And another strategy could be that of including in the same CAR construct the SCB for the antigen of interest, together with another part specifically addressing the tumor microenvironment, and in particular, FAP could be a good alternative to be targeted together with GD2, exactly for the reason of simultaneously attacking both the tumor elements and the microenvironment supporting the growth and the progression of the tumor cells.

Dr van Ewijk: Thank you very much. And I'm on the roll. In the patients with the osteosarcoma issue, I think it's quite impressive to see such a good response. How much GD2 expression would you consider enough to include such a patient for CAR-T, or was there any specific level of expression, or any level of expression?

Prof Locatelli: For neuroblastoma, we didn't establish any threshold. For the bone sarcoma, we required that at least 1/3 of the tumor cells express GB2 for being eligible to be treated. And that, yeah, certainly appreciated from the slide that I presented that the clinical response that can be obtained through the use of a GD2 specific CAR-T cells, in both Ewing and osteosarcoma, is very impressive. So, in principle, these bone tumors can become another type of neoplasia suitable to be treated with the GD2 specific CAR-T cells. But what I like to particularly emphasize is that now, in view of these results, it's important to have the courage, and let me use this term, to anticipate the use of CAR-T-cells in newly diagnosed patients, because the safety profiles, as has been demonstrated, that none of the patients we treated experience a fatal event related to toxicity. And considering the good safety profile, and the results that were better in patients with a low tumor burden, my carcinoma view is that of anticipating the use of CAR-T cells in earlier phase of the disease.

Dr Perez: Thank you very much. And one, I think, last question. How long will you think, if this continuous succeeding, this will be available for a bigger trial, are the center trial?

Prof Locatelli: So, we are closing. That's a very important question and thank you for asking. We are closing now our non-institutional trial, and we want to continue now to launch a multicenter trial, and we will be more than happy to collaborate with any institution potentially interested to work with us to further validate the approach on a larger cohort of patients, and also, in children with much less advanced disease. Thank you.

Dr Perez: Thank you very much.

Dr van Ewijk: Thank you very much for your talk and discussion.

Prof Locatelli: Thank you.

Dr van Ewijk: Thank you.

Prof Locatelli: It was a great pleasure to interact with both of you. Thank you.