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Neuroblastoma in childhood

Prof Ladenstein: So, welcome very much to this early evening or late session today. And we are more than pleased to talk about neuroblastoma, as two ladies who have been in the field for quite a long time. I allowed myself to give a little bit of a subtitle. Because neuroblastoma is one of the very fascinating tumours, and you could call it a chameleon in paediatric oncology because it behaves sometimes quite differently from what it appears to be on a first sight. So, as an introduction just to remind you, all your views are important and just remember that you can ask your questions anytime and send your comments in any time. And please do so. We will pick up your questions from the chat and we'll have an open discussion at the end of the meeting. But you are allowed to interrupt and then, questions will be addressed, and we will try to respond in a quite interactive way. So, I start now the lecture. And this is just to recall to you, the basics of neuroblastoma. It is a tumour of sympathetic neural crest origin and is most often located in the adrenal gland. Occurs in very young children. The median age of occurrence is 22 months. And to guide us in the whole scope of paediatric oncology, it's the most frequent paediatric extra-cranial solid tumour, and accounts for about 8-10% of the total incidence. So, today, we differentiate two major risk groups. Low and intermediate-risk neuroblastoma which have roughly, altogether, a very good event-free survival outlook with the ongoing strategies. And this is one of the major breakthroughs during my professional lifetime, that in these risk groups, a lot of de-escalation was able to take place. And on the other hand, the group of high-risk neuroblastoma is still a group that worries us because if you look at long-term outcomes, we come to roughly 40% event-free survival only, in spite of very intensive strategies. And we are undertaking major efforts to improve this towards the better. And we will come back to the details, later on in the session. So, first of all, basic standard diagnostics up-to-date. The first orientation, when a patient presents with clinical symptoms is certainly to investigate with tumour markers. One of the hallmark tumour markers, easy to catch are urine catecholamines, but also, in the serum, you can orientate yourself with neuron specific enolase with ferritin and LDH to get some orientation. But very clearly, a first ultrasound followed by a CT or MRI is really a standard. And what is not so easy everywhere in Europe, but still, international standard, is to undertake an MIBG-Scintigraphy. In the lower part, of the slide, you just can see how such a scintigraphy shows the uptake, particularly, in metastatic sites. The heavy involvement throughout the skeleton. MRI teaches us the tumours and their extent. And you see on these examples on the right-side, one of the peculiarities, we have quite often a very large primary tumours in the abdominal or retroperitoneal region as the most frequent one, but not the only site. You see the complexities of extension particularly, towards the spine, and the invasion that gives special problems in terms of paralysis depending on the exact location of the respective primary tumour. Histology is key, more than ever, and we aim for sufficient tumour material on first biopsy, because tumour genetics are absolutely fundamental these days to direct oneself in the right way. In metastatic disease, if there is heavy bone marrow invasion. Excuse me. Then, there is a possibility to get the major diagnostic hallmarks from a genetic point of view out of the bone marrow. And one potentially could spare, in a patient that is in poor clinical state, the open biopsy. Although, the overall theme is with the way we want to move the field forward, we

aim for tumour material at diagnosis and later time points to better understand what's going on, and to improve the treatment options. So, bone marrow to aspirates, to trephines, are the common standard. And it's probably worthwhile just to give you here an oversight how this presents. Typically, neuroblastoma is a solid tumour in the bone marrow plus distends in clumps. And it's once seen, a quite re-connectable presentation, also called Homer-Wright rosettes, under investigation in the microscope. If we look here at genetics, probably, two messages, I want to pass here on to guide us into the field. We have two major pathways. One allows neuroblastoma to go into spontaneous regression or differentiation. This is the pathway shown on the left-side when you look in the screen. Typically, triploid tumours. Typically, no segmental chromosomal aberrations. And here, is really a way to get a cure with very little interventions. I have put here, thoracic neuroblastoma, in this way occasionally detected and it's usually mature tumours. Sometimes, can be home of resection surfaces, sometimes detected later on in life as such. But there's a completely different pathway where we deal with highly aggressive tumours which are prone to progression. And our major hallmark here is MYCN amplification. It's one most important stratifying risk factors which can be found in quite a high distribution but depends a little bit on age and the percentage you find in the overall cohort. But other important hallmarks are chromosomal deletions and here, particularly, 1P36 deletion, and chromosome 11Q deletion are the important ones that are stratifying. And behind are also further genetic aberrations like TERT-translocation, or ATRX, for example, that give you a signal towards adverse outcomes. So, there is a lot of background already in the SIOPEN group which came together meanwhile more than 20 years and is a common interactive group, with a reach out beyond Europe. So, we are the international SIOP Europe Neuroblastoma group. And the first study that we were running in the low-risk segment, was the LNESG1 study published in the British Journal of Cancer in 2008. And you see on the right-side, on the survival curves, these really impressive outcomes in the majority of patients particularly, for LNES stage 1, and a little bit less favourable, also for stage 2 patients. And on the left side, plot A is event-free survival and plot B is the overall survival. So, even if a relapse occurs, a high percentage of these patients can be rescued. And what we have shown in terms of a treatment strategy was that surgery alone was effective and a safe treatment for localized and resectable neuroblastoma, with these excellent outcomes. And it was important that no MYCN amplification is present. However, we also have seen that patients with stage 2 that have an unfavourable histopathology and elevated LDH, suffered quite a high number of relapses but the majority of those could be rescued. And MYCN, I already underpinned, that this really drives relapse and must not be overlooked in the diagnostic procedures. So, this is a very busy slide. And I refer, or I suggest, that you have a direct look into the JCO publication of 2020 because it had some very interesting findings, and it was a cooperative work done between SIOPEN and COG. And we were here very much interested in the genomic analysis of localized resectable neuroblastoma from major trials, from Europe and North America. And what was the finding here? That age really had an impact with a cut-off of 18 months and the presence of segmental chromosomal alterations, particularly 11Q loss, significantly reduced the survival in the older patients over 18 months, but not in the younger infant or 12 to 18 months patients. So, overall, the big circle, very favourable outcomes in both trials, LNESG1, LNESG2, and the COG data. And on the right-side, you can see here in greater detail, the unfavourable outcome in the older group if you have segmental chromosomal aberrations present. And this is again shown here in the third red circle, the impact of 11Q in this older age group, but no difference in the younger ones. It is very important to recognize that there has been a major international collaboration to fine tune and improve the staging system. The report on INRG is published here, for example, in JCO 2009 and what was important or the intention to bring in an international neuroblastoma risk group staging system, replacing the international neuroblastoma staging system, INSS, was to have a common language that is standardized at the moment of diagnosis and is a pre-surgical one. So, we have here really a judgment in the localized disease patients that the tumour that is not involving vital structures, and there are certain image-defined risk factors who determine this definition. As opposed to those patients that have locoregional tumours or with presence of one or more image-defined risk factors. Then, we have instead of stage 4, stage M. And for the 4S, the definition of MS, I think that's quite important to keep in mind to use this common language, and it's based on the current standards. It might be interesting

for the slides to follow that in SIOPEN, in the trials, particularly, also in high-risk neuroblastoma, we were using still the age cut-off of 12 months. And this has reasons with outcome results, but I will highlight this whenever necessary. And it's also interesting to say that infants with stage M or MS are low-risk, but those with only metastasis demonstrated by CT, or lung and pleura, or CNS metastasis, are really in the intermediate-risk category. This just shows you in an oversight, all the factors that intervene into risk grouping. So, apart from stage, age is an important factor, but also, histology intervenes in the description if it's neuroblastoma, a ganglioneuroblastoma, particulate at category, ganglioneuroblastoma intermixed, or maturing, or mature ganglioneuroblastoma. The grade of differentiation has an impact and MYCN and 11Q employed intervenes, and this gives you all the series of risk group that allows us then to compare internationally the respective categories. Even if patients have been treated by a different treatment philosophy. In SIOPEN, the low-risk group is described by L1 neuroblastoma without a MYCN amplification, or children under 18 months with L2 tumours, no MYCN amplification, the infant group with M or MS neuroblastoma, no MYCN amplification and included also the neonatal adrenal masses that are MIBG positive. The intermediate-risk group, then, are those patients that present with L2 tumours in the age group over 18 months but without MUCN amplification but associated with image-defined risk factors. Then, the stage M, under 12 months which involves bone, pleura, lungs and/or the CNS, but no MYCN amplification. And last not least, localized resected neuroblastoma, former stage INSS1, but with MYCN amplification. So, altogether the treatment recommendations are based on an algorithm that combines age, stage, genomic profiles, and the life- threatening symptoms. So, ultimately, that's under the discretion of the respective clinician to judge on the life-threatening symptoms, but a clear guidance is given on what drives interventions. That could be in the case of intraspinal neuroblastoma, that could be pain, major gastrointestinal symptoms, respiratory, cardiovascular, renal, hepatic, or bladder/bowel dysfunction, et cetera. The spinal cord compression is something I want to highlight because it's so specific to a neuroblastoma. It's considered as a life-threatening symptom in LINES, and for the low-risk neuroblastoma guideline. In both symptomatic and asymptomatic patients, there's a recommendation to start urgently chemotherapy with the VP-16 and carboplatin. And in symptomatic patients, we are confronted with pain and potentially irreversible loss of neurologic functions. But also, we recognize asymptomatic patients, and this is a concept of spinal cord involvement, needs treatment, but a less aggressive immediate intervention. So, symptomatic patients are an emergency, and they are subject to either urgent neurosurgery or chemotherapy plus high-dose glucocorticoid. And is usually subject to discussion with your neurosurgical team. Opsoclonus Myoclonus Syndrome is also a very distinct phenomenon because almost 50% of these children have an underlying neuroblastoma. This is an important phenomenon to be recognized. It's considered a paraneoplastic syndrome. It's altogether a rare presentation, but a clear guidance. And we have been running a specific trial and treatment recommendations on these ones which we can help out when you encounter this. The study is currently closed and subject to publication. So, it will be then widely accessible. Some words about LINES, it's our European low and intermediate risk neuroblastoma study currently ongoing, where the study lead is Adela Cañete from La Fe. It's a number of countries that participate, in total 15. And what you can see on the right-side, are being a product of a common study committee, planning meetings, and having established all the necessary interactions with establishing a DMC, biology group, radiotherapy group, pathology, and national coordinators. It took a while to bring countries onboard, but ultimately, it's a strength of the group that it happens. This is as far as I want to go into treatment details. This is the protocol at a quick glance. What we can see in the low-risk group, basically, that just in the MS group under 12 months, no life-threatening symptoms. We go into observation only. We had a question on L2 tumours under 18 months, no life-threatening systems to have a randomization versus just using cyclophosphamide and vincristine. And in the other groups, you have a start and kick off with VP-16 Carbo and CADO, and you evaluate every two cycles if the patient is ready for surgery, that could indicate the treatment stopped at a given moment in time, or you continue further with cycles and changing gears to so-called CADO cycles, cyclophosphamide, adriamycin and oncovin, and then, aim for surgery as a success of these treatments. If we look now into the intermediate-risk groups, a very similar strategy, basically,

depending and taking into consideration the histology but, importantly, in group 8 with poorly or undifferentiated neuroblastoma on top, you have radiotherapy coming into the game, and then maintenance. And we have similar strategies also, for the group 9 and for the group 10, where you can add-on chemotherapy up to 8 cycles in total. So, just to remember, you can ask questions, anytime if you want. And now, we switch gears to high-risk neuroblastoma. We have an international collaboration but sometimes, slightly different defined risk groups. That's why I show you here both, to highlight some differences between COG trials and the SIOPEN trials. In our last high-risk trial, we considered patients over the age of 12 months with metastatic disease to be high-risk, but also, all tumours MYCN amplified at stage 2 or greater. And also, patients with a stage-4 have shown MYCN amplification. In the COG, slightly different, because here histology and diploid DNA content has an additional impact to categorize a patient into the high-risk features. And they are really in the 18 months cut-off in the strategies and advanced stage diseases of the previous INSS, with certain conditions, would also be eligible for an intensified strategy. On the right-side, this is the COG outcome results on more than 2000 patients, roughly 30%, under much more favourable outcomes in the low and intermediate-risk. And this is a little bit the situation, as we see it also in SIOPEN with slightly better outcomes with a younger cohort in the high-risk group, in our ongoing and thus, more recent trial. How did we achieve results? So, I will guide you quickly through a series of randomized trials that were building the knowledge. Mainly, really focusing on the survival curves to tell you the story. You can read then in these abstract summaries later on, when you revisit the session. But the first fundamental randomization pursued by Jon Pritchard was to test high-dose melphalan in the setting of high-risk neuroblastoma. And those who achieved a complete remission or a good partial remission after opioid induction and surgery had a better and improved event-free and overall survival. So that was the first step towards high-dose treatment. And these days, we are very much aligned that we use multi-agent chemotherapy induction. We plan in this period for surgery and for stem-cell harvest. We know that consolidation has a major role. And we obviously, since quite a long time use peripheral stem-cell re-infusion rather than bone marrow transplantation, which has improved largely the toxicity scale of the transplantation. And in the post consolidation phase, more recently, after isotretinoin was established through the COG, the breakthrough publication by LSU establishing Anti-GD2 antibody-based immunotherapy as a major impact and improvement in high-risk neuroblastoma. Shifting the survival outcomes in the defined groups of patients having achieved a complete remission up to 60%. What else were major impacts? If we look here into a randomized trial of myeloablative therapy, a hallmark study by Kate Matthay, 2009. It's very clearly shown in these outcomes and in these event-free and overall survival curves, because very clearly the red line was intensive chemotherapy only. If you add 13-cis-RA, the blue line, you're doing slightly better. Bone marrow transplantation without 13-cis-RA further pushed the curves up. And apparently, the yellow curves show you the outcome of bone marrow transplantation plus 13-cis-retinoic acid. And in overall survival, you see the results, and the strategy that has become a standard of care currently. What is also interesting to underpin are the results of the German group that investigated high-dose treatment versus maintenance chemotherapy. And have shown a better outcome in the high-dose chemotherapy group which you can see here with the green curves, event-free survival, overall survival, significantly better. Purging is another question that is of a major interest that was raised. At what time should we purge? And the conclusion from this major COG trial was that we could not discover a major impact on purging. That's why the conclusion was that non-purged peripheral stem cells were acceptable to support myeloablative therapy in high-risk neuroblastoma. This just gives you in an oversight on the right-side, overlapping curves which were this proof why a strategy of non-purged peripheral stem-cells is testified, published by Kreissman in Lancet Oncology. And to the left-side, you just see the basic cornerstones that I previously outlined regarding the treatment strategy. Then, more importantly, a major hallmark study by Julie Park published in JAMA 2019, was really about the effect of tandem transplant that showed a significantly better survival than event-free survival. But some findings were discussed as needing some more investigation. But nevertheless, this is the key-message from this trial, that the tandem transplant here had a superior outcome. And although the overall survival was then quite similar, the event-free survival was

prolonged through the tandem approach. And most importantly, the COG also used immunotherapy. And in this very transplant group, this effect was even more pronounced. So, again, think about questions. We will be ready soon. Now, I'm coming to the SIOPEN strategy. We have in the trial more than 3,500 patients, 5 randomizations that we dealt with, 4 treatment standards were established, and 14 publications on this trial to-date. So, this is for further reading. We clarified that adding G-CSF in Rapid COJEC, in the induction regimen, significantly lowered the toxicity profile of this regimen, given every 10 days. More recently this year, we published this R3 randomization, where we compared against this modified N7, actually, the N5 regimen. And from toxicity profile here, Rapid COJEC was the winner for us. We also compared the high-dose treatment concepts of our European established regimen of busulfan-melphalan comparing with the same regimen as used by the COG group. In our hands, busulfan melphalan is the superior one, and therefore, SIOPEN standard. And then, we also raised questions regarding the immunotherapy, and I'm coming then back to this, isotretinoin is in all these strategies. And we had first a short-time infusion schedule, and then, swapped for toxicity reasons to a long-term infusion schedule. And we ultimately concluded that it's better not to use IL-2 in this setting. And our standard currently is a dinutuximab beta, immunotherapy addition to therapy on its own. It was a long story to develop this drug, that I don't have the time to tell you in detail. But this very close collaboration, ultimately ended in an approval of EMA in May, 2017. And to make this drug available, as it was started with fundraising help of parents' organization charities to produce the first lots so, that we could treat patients on the trial. So, it started investigative driven and resulted in a very successful drug accessibility on the market at the end. So, this is just showing you in brief, the superior outcomes of busulfan-melphalan over CEM as a high-dose treatment. You can have details in the Lancet Oncology publication of 2017. So, we gained roughly, depending on event-free or overall survival in these settings, 15%. And dinutuximab beta, in the randomization 1 with the short-term infusion, roughly 20% in event-free survival and overall survival. Quite long observation times, and also, published in respective journals, in Cancers, but also, in Lancet Oncology. What we also were able to show is that you could think this is a negative result, but for us, a very important result that the combination in terms of event-free and overall survival was not better. And this is true for the short-term setting, as well as for the long-term immunotherapy, dinutuximab beta infusion setting. And this is a very important message to deliver effective treatment, but in a less toxic way. Not to forget in multidisciplinary teams, that our surgeons have a major role in the overall treatment. And we could show in this publication, in JCO, that complete microscopic excision results indeed in a survival advantage, and this was true in the pre- and in the post-immunotherapy era. This is just recalling what I pointed out previously. R3 randomization has produced overlapping results, and we decided for Rapid Cojec because it proved to have a less toxic profile as compared to the N7 ans SKCC regimen. And a very recent publication underpinning how important it is to have bio-sampling that you can go back, and we did this with the biological group to investigate ALK amplification, and clonal mutations. And you can see that here we have a major impact if we find these changes, and this is actually driving and impacting the current high-risk two trials in quite a way. But here, I leave it then to Dr Valteau to comment. Where are we heading in neuroblastoma? In principle, we were interested to compare with the German group unifying with SIOPEN to compare the German induction with Rapid Cojec, to be sure about our standard of care induction. And we are asking here because our setting is different as opposed to the US, if busulfan alone is doing as well as going into a tandem setting by adding high-dose thiotepa. We have a radiotherapy question. This was very important for us of asking in a randomized way, if a higher-dose here in residual disease would then improve the outcomes, we go with the standard of care of dinutuximab beta. And ultimately, for those patients that are refractory, or poor responders, in the front-line setting, they enter the VERITAS protocol, where again, we shift patients into this trial. They're receiving courses of haemotal and irinotecan. And then, a question on the better double transplant, either using MIBG or high-dose thiotepa followed by busulfan-melphalan local treatment, and standard of care maintenance. So, this is just in a nutshell, all the relapsed/refractory cell setting where we established the long-term infusion schedule on dinutuximab beta. And we later then also asked the randomized question of adding, yes or no, IL-2. I think there are a lot of learnings within these trials that we can taper off morphine usage, which is needed when

you start out with immunotherapy, particularly, with the long-term infusion setting, but also course-by-course. This is a quite promising outcome that we are seeing in event-free and in overall survival. And in particularly, I raise your attention to the quite high response rates that are observed in these relapse/refractory settings at the end, with 43%. We are aiming to integrate, ultimately, immunotherapy in the front-line setting. So, this is in a scaling-up phase, and hopefully, coming soon, and building on previous experiences. So, there's not much more to say. So, now, concluding, I think we have to take some take-home messages here, that neuroblastoma truly is marked by a very wide clinical and biological heterogeneity, that sometimes, internationally, is rendering comparison across respective trials referring eligibility and treatment intensity as slightly difficult. But nevertheless, the groups are inspiring each other and we all together try to improve the treatments. And an output over the last four decades is the big learning between the low and intermediate-risk groups with the de-escalation strategies and the intensification strategies in the high-risk group. I think that the hallmark randomized trials, I pointed out, that clarifies very much how important international collaboration is, and constantly building on creating evidence. I think we are not yet there. We are encouraged by what was achieved in acute lymphoblastic leukaemia. So, my wish would be to see similar outcome rates in high-risk neuroblastoma, but there's definitely a way to go with a lot of innovation needed and coming in. So, the exciting times that will be coming up, and I think continued collaborative efforts are absolutely key. And obviously, it's not survival, but we are also very much interested in improving the quality of survival, reducing toxicity, and to minimize the late effects. So, thank you very much for your attention. And I'm handing over now to my discussant, Dominique, to manage questions or to trigger some discussion. Thank you.

Dr Valteau-Couanet: Thank you very much, Ruth, for this great talk covering the wide landscape of neuroblastoma. At the moment, there is one question concerning the surgery and the completeness of surgery, its impact on the survival and its impact on our strategy. Can you answer this question about, when surgeons are not able to perform complete surgery, what is the impact? And maybe the answer is different according to low, and intermediate-risk, and high-risk patients.

Prof Ladenstein: I think most importantly, a point to make is when we are in the high-risk setting, learnings that I couldn't touch on was that if the tumour is not resectable or easily resectable, or only at the price of nephrectomy prior to high-dose treatment. We postpone it to the setting after high-dose treatment because in this particular patient cohort, the systemic treatment overrules the local treatments, so that we have really good metastatic control. And we have seen that there is no disadvantage if you postpone the surgery but nevertheless, we aim for resection. But we all know that there are some cases in neuroblastoma that are simply non-resectable, and that underpins the important role of radiotherapy to achieve local control. And that makes the question so interesting that high-risk 2 is raising. If a higher local dose improves the outcomes, particularly, in those patients where there is a post-operative residue or a completely unresectable tumour, as we all know such cases exist. But it's worthwhile to aim to go to a highly specialized centre's skilled surgeons, because it's a very specialized intervention and needs a lot of experience that produces the better outcome results. I think the attitude in low and intermediate-risk is quite different because as I pointed out, when you are under the phenomenon of where a patient potentially could have either a maturation of the tumour or a regression of the tumour, surgery does not play a major role, so, there is time. But importantly, you have to clarify the genomic profile of the patient which then drives the necessity of an intervention at a given moment in-time. Obviously, the unfavourable histopathologies, here also have a factor, most likely on top of the genomic ones that I pointed out, that in L2 traumas play a role. So, I think that's probably at large addressing a little bit the diversity of discussions that we need to have regarding surgery in neuroblastoma, overall.

Dr Valteau-Couanet: Thank you very much, Ruth. I think that one important point is the fact that in high-risk neuroblastoma, the local treatment has an importance in the survival at the end. So, even in the metastatic disease in these high-risk patients, local treatment is really important. The reason why we have an excellent

collaboration with surgeons and radiotherapists, is to try to go for the best local treatment. There is another question, Ruth, about dinutuximab beta. An interesting question about the doubt of the benefits of dinutuximab beta in patients without residual disease. And the question, what is guiding our strategy?

Prof Ladenstein: I think this is a brilliant question. Thank you very much, I just love it yeah. Because what you should know is, the results that I have pointed out today that were published in the New England Journal of Medicine by LSU. These are the results in patients in complete remission prior to immunotherapy, and here, you have the added benefit of 20% in a randomized setting. So, I think this is a very clear answer. You don't need to have any doubt about the effect of dinutuximab beta in CR patients, yeah. And we are showing the same effect in our publications of SIOPEN. Yeah, so, go ahead and do it. It's really beneficial, yeah. Evidenced.

Dr Valteau-Couanet: I think that I completely agree with you, Ruth, because clearly what we have done during the two last decades is to show that now treatment of high-risk neuroblastoma patients had four phases. Induction treatment aiming at controlling the metastatic disease. Local treatment with surgery and radiotherapy. Consolidation with high-dose chemotherapy, and with autologous stem-cell transplantation. And this maintenance treatment with immunotherapy, it changes things a lot. There is another new question. The question is about the benefits of increasing Tru-cut biopsy over open biopsy, that Tru-cut biopsy could obtain less material and could impair the tumour analysis, and to impair to find the prognosis factors?

Prof Ladenstein: I think depending is a Tru-cut biopsy bringing in enough material is an ongoing discussion. And I think it depends very much on the skills of who takes the Tru-cut biopsies. You know that one sample is not enough. If you really sample from three to four sites as it is advised for tumour heterogeneity, then probably, you're even more safe with an open biopsy. So, I think that's a little bit left to the discretion, and I think your biologists will give you very good feedback. And the pathologists, if what you produce, is sufficient material because this is quite site-dependent. It's an ongoing discussion, but if it's judged as sufficient, that's basically fine. But we encourage more and more open biopsies because there are so many questions to be answered and you really need to have enough material, not only for the routine diagnostics but also, for forthcoming research. Obviously, material gained at diagnosis is vital and very important and needs to guide us. I think the post induction tumour, this is interesting, but we know that tumours are reacting to drug exposure, building-up resistance, sometimes changing some pathways. So, I think characteristic major hallmarks might or do persist, but nevertheless, we have here a changing profile that we are interested in. And ultimately, I think, we may say, but this is subject to ongoing research right now. This will be complimented and is complimented by liquid biopsies where we increase our understanding and fine tune the techniques so that they, in the future, will become more reliable. But we are not yet there. And I think we are in an area of high research opportunities where bio-sampling, material sampling, is absolutely key. So, that in the next two centuries, we have more breakthroughs with new drugs.

Dr Valteau-Couanet: Thank you. And as I said, probably in the future, circulating-DNA could help us to follow the tumour and its modifications, with time. And maybe, we could hope to avoid any biopsy, also, at diagnosis, if it's really shown to better display the heterogeneity of the biology of the tumour. There is no more questions in this chat but I have a question, Ruth, about the prognosis factors that have been identified in addition to MYCN. And behind that, I have two questions. First, in events there is a MYCN, isn't any more a prognosis factor? And the second question is, what have we shown in our treatment to be a prognosis factor for these patients?

Prof Ladenstein: I think MYCN is still a major stratifying factor when you're at first diagnosis.

Dr Valteau-Couanet: Yes, of course.

Prof Ladenstein: I think, that's the most important take-home message. Quite interestingly, with the intensive treatments, we more and more see that the different biological profiles somehow impact on the response behaviour, one way or another. So, our MYCN is still a major factor in localized tumours or

locoregional tumours. And obviously, as I pointed out, in the infant population, it has a major impact. But in the patients stage-4 metastatic, over 12 months, in our hands, it does not discriminate any longer the populations under the current very high intensive treatment concepts. And I think my answer would be yes, still an extremely important factor. And when we get requests in countries where, for example, MYCN is not a routine, we see a lot of under or over-treatment in both directions. So, I think to have this established and have it accessible is still fundamental. And we will see over time, and this is not new, that under a change of treatments and very successful treatments, you might change the profile or the hierarchy of your important prognostic factors. Also, in the biological ones.

Dr Valteau-Couanet: The point I wanted to underline is the fact that, of course, it's a prognosis factor, and patients with localized disease with MYCN amplification have to be treated with high-risk treatment. But what we have shown, and you have published too, is the fact that using in such patients, a high-risk strategy could allow them to have a much better prognosis. And patients with localized disease with MYCN amplification as in advance, have good prognosis. And patients with a stage 4 neuroblastoma and MYCN amplification have not a poorer survival than those without an MYCN amplification, which means that the treatment is overcoming the biology. But of course, we have to take into account this biology to treat the patients.

Prof Ladenstein: Well, excellent for this summary. That is roughly what I was trying to say but I think you made it now much clearer.

Dr Valteau-Couanet: Sorry.

Prof Ladenstein: What we had tried to bring across. No, thank you very much.

Dr Valteau-Couanet: And...

Prof Ladenstein: No worries.

Dr Valteau-Couanet: And there's a point, the impact of those tumour metastatic situations at the end of the induction treatment, which is, as you have shown it too, a major prognosis factor. It's the reason why we want to improve this response for a better statistic response rate and adapt the treatment behind it if the response is too poor. There is another question I have seen. I've seen another question, which is the fact that concerns about the treatment posed between high-dose chemotherapy and the local radiotherapy. This person wanted you to comment on that.

Prof Ladenstein: I think maybe, you even can do it, as chair of high-risk too, because I think it's really under your wings and I already have talked too much. So, feel invited to answer this question, Dominique, please.

Dr Valteau-Couanet: So, at the moment, we have a delay of at least 60 days or 70 days between high-dose chemotherapy and radiotherapy. And this was proposed because we are concerned by the potential toxicity of radiotherapy after the busulfan administration. We know that busulfan can increase the toxicity, and we may have acute toxicity or severe acute toxicity, because we are performing radiotherapy too early. It's the reason why we have such a delay. Sometimes, because of the toxicity of the high-dose chemotherapy, we have to postpone a little radiotherapy because of the general toxicity. This is another point that has to be taken into account, and we have to manage that. And, of course, with a risk, when the delay is too long, to have disease progression at that time, but it's not so often at this point. I think of the crucial delay concern we have, is the time between the end of induction and the beginning of high-dose chemotherapy, especially in patients with MYCN amplification. Because these patients can have a rapid and brutal progressive disease at that time if we postponed to match the general treatment. It's the reason why we have to be very careful, because at that time you have to collect stem cells, perform surgery, and we have to be careful in these patients with MYCN amplification to try to perform the high-dose chemotherapy as soon as possible. Sorry. And the timing is very important in these patients.

Prof Ladenstein: I'm just looking at the time, and it seems that we have reached the end of the allotted time because it's now 07:15 PM. And I think we were told by ESO this is about the red-line where we should stop.

Dr Valteau-Couanet: I think that we have answered all the questions that were raised. And so, I thank you very much, Ruth, for this great talk. And I hope the students are happy with this, that we have answered almost all questions.

Prof Ladenstein: Thank you, Dominique, for being a brilliant discussant. And we wish everybody a good evening. Thank you very much. And don't forget to get your CME points, answering the questions. So, that makes... this is the good thing to do for your curriculum and for your learnings. Thank you very much for attending. Bye-bye.

Dr Valteau-Couanet: Thank you, bye-bye.

Prof Ladenstein: Bye-bye.