Frédérique Penault-Llorca
Today, with Andrea we are going to go through the diagnosis and treatment of *NTRK* fusion-positive GI cancers. Andrea, what are the *NTRK* genes, and what about the *NTRK* fusion; why are they so interesting to look at in GI cancers?

Andrea Sartore-Bianchi
Yes, thank you Frédérique, and indeed we know the gene fusions can be a driver of oncogenesis, but it is now when we are seeing very important advances in clinical sequencing technologies that we are aware of new unknown drivers, that continue to be discovered across a variety of cancers including GI cancer.

So, some of these drivers are indeed recurring gene fusions, and this can involve the *NTRK* genes, that are present across multiple cancer types and very importantly, from a clinical standpoint are amenable to pharmacological inhibition. In particular *NTRK*1 was first identified as an oncogene in the 80s, and this actually happened exactly in a tumour specimen from a colorectal cancer. Now, we know that the family of TRK receptor consists of three receptors - TRKA, TRKB, and TRKC.

These are transmembrane proteins. TRKA is encoded by the *TRK1* gene located on chromosome 1, TRKB on chromosome 9 and TRKC on chromosome 15. Importantly, each of these TRK receptors consist of an extracellular domain that can be inhibited indeed, by a specific drug, a transmembrane region and then an intracellular region containing the tyrosine kinase domain.

The TRK receptors are activated by a family of four proteins that are called the neurotrophins. And these play an important role in the development and function of the nervous system including memory formation, and retention, nociception and also proprioception. Now, what happens is that in some tumours these NTRK proteins can be altered because there are gene fusions that can take place, with a region of the *NTRK* gene becoming joined with a fusion partner gene, either by intrachromosomal or interchromosomal rearrangement. So, the result is a fusion gene encoding a protein with a constitutive tyrosine kinase domain activity, that is ligand independent and therefore has interrupted downstream signalling messages that promote cell proliferation and survival. There are many fusion partner genes identified so far, but what is important is that the fusion protein can be, again, pharmacologically blocked by a specific inhibitor to treat our patients.

Frédérique Penault-Llorca
So, Andrea, if in the lab we have a positive result for an *NTRK* gene fusion, so *NTRK1* or *NTRK*3, what are the treatment options for those patients, today?

Andrea Sartore-Bianchi
We have several *NTRK* inhibitors, some are in development. But most importantly, we have two of these that are approved for treatment of *NTRK* positive cancer. And these independent of a tumour type, in what we call the histology agnostic indication. So, larotrectinib has been the first in 2018 to obtain this indication. And it is a potent and selective inhibitor of all three TRK proteins. And we have also entrectinib that obtained

approval in 2019 by FDA. And also, this is – both drugs are oral inhibitors and also entrectinib is oral- inhibitor, this is a pan-TRK inhibitor or all TRK proteins with some additional activity also against the *ROS1* and *ALK.* So, these are the drugs that we can use to treat patients found positive with *NTRK* fusions.

Frédérique Penault-Llorca
But the problem for me is that those fusions are extremely rare. And it's very complicated to be sure, that we don't miss any patients. So, we have several methods that have been developed to find TRK fusion-positive tumours, one of the methods will be immunohistochemistry. Because when we have these fusion genes, we have an increase in the protein expression. So, it's something that can be implemented locally in any lab, but it should be a reflex test, and it's not always possible in terms of resources, and it's also something that pathologists have to keep in mind. Then we can look at the rearrangement by FISH. Here again, it's a technology that is implemented in many pathology labs, but it's not a reflex test, *per se*, for *NTRK* in those tumour types, but is something that we use in specific tumour types like secretory breast cancer, or mammary analogue salivary gland because it's part of the diagnosis but for the other type we are not using FISH so frequently.

We have other possibilities with RT-PCR, for instance, where we have already some tests that have been designed, but they are limited by the partners that have been chosen to look at *NTRK* so we can miss some of those extremely rare alterations. So at least we need to look at *NTRK1* and *NTRK*3 because we know then that *NTRK2* is extremely rare. Alterations in *NTRK*2 are mainly in the central nervous system. And then we are going to look at the more frequent partners we, as I said, can miss some of them and so that's why NGS is for me, the best technology and then it will depend for colorectal cancer if in the routine and in the reimbursement policy of the country, NGS is reimbursed or not for colorectal cancer, because we have already several alterations to screen for colorectal cancer: RAS alterations, BRAF, looking at MSI, looking at HER2.

So maybe, at least in my institution, we use NGS for those patients. But if we don't need NGS, maybe we will have to do some triaging, at least for colorectal cancer, because we have some data now showing that in colorectal cancer, probably the overall prevalence of *NTRK* fusions is extremely low. It’s probably around 0.25-0.5 but not more than that. And, but what we know is that it's more frequently in the right colon. It can be found in tumours with poor differentiation, mucinous or signaturing cells or solid patterns. So, we have some cases like that. And probably the most important is that it's more frequently found in the tumours with microsatellite instability. Also, the *NTRK* gene fusions, they are usually found, in cases where we don't have other gene alteration, rival alteration. So, they are exclusive, supposedly exclusive of RAS alteration or BRAF alteration.

So, we could think about a kind of triaging using a way of an exclusionary testing, screening for *NTRK* fusions in MSI high, dMMR and BRAF wild-type colorectal cancer, for instance. And here we have data showing that, in this kind of population, we can have up to 15% of *NTRK* gene fusions.

So, this could be a way of looking at that, and it's already in some algorithm like the recently published algorithm by the Canadian Oncology Group. And with this strategy, you are only

going to narrow the population to screen to maybe 5% of metastatic patients. So, RAS, BRAF wild-type, MSI-high and dMMR. And here, maybe we have a better chance to find that.

But from my personal experience, we had cases, MSI-high, BRAF mutated with *NTRK1* gene fusion. And we also have one case with KRAS mutation. So, if it's possible, if we have enough resources, I think it could be interesting to consider *NTRK* fusion testing also, in microsatellites stable, pMMR, RAS/BRAF, wild-type patients, but it's a larger population, but it's only if resources will permit it.

So, Andrea, what is your point of view? Do you recommend testing in the MSI-high group? Or do you think we also need to enlarge to the other tumour type, what are you doing in your clinic every day?

Andrea Sartore-Bianchi
Yes Frédérique, indeed, as you said that these are very rare alterations to find and the thing of having now an enriched population that as you said, is MSI-high, RAS and BRAF wild-type is very helpful. So, what we are doing is to test for *NTRK* all patients in this category. Of course, it would be desirable also to have data about all other cancer patients and from this point of view, you know, having an NGS testing at the beginning of the therapeutic history can be helpful. Of course, there are some barriers for reimbursement of having NGS in all of our patients, but I think that this is something that we have to implement in the future, also for GI cancer, especially colorectal cancer.

Also, from a diagnostic point of view, I think that also liquid biopsy will be in the future something helpful because we know that at least we can monitor treatment with *NTRK* inhibitor through this method. Of course, this should be regarded so far as experimental and to be developed in clinical research but again, as for all targeted therapies, I think that also liquid biopsy can provide clinicians with important information about mechanisms of resistance and how to overcome these mechanisms.

Frédérique Penault-Llorca
Andrea, with two drugs being available to target *NTRK* fusion-positive colorectal cancer, what do you do in your practice? What are the data today that we have for those tumour types?

Andrea Sartore-Bianchi
Yes, and we know indeed that we have effective inhibitors, and we have you know, data of entrectinib and larotrectinib in all tumour types in the histology agnostic indication. So, for example, we have data of larotrectinib, from the joint analysis of phase one, two and three trial in paediatric and adult patients. And we know that with this treatment, you can achieve high response rates 75% and durable responses.

Now, what is interesting I think also for the purpose of this podcast, is that we have now updated data regarding the GI oncology space, because at the recent World Congress on Gastrointestinal Cancer, updated data had been presented, from this trial, joint by Dr. Boni and the recent analysis in 18 patients with the GI tumours, so the majority were colorectal cancer and as we noted before, these were mainly MSI-high patients, so 7 out of 10 were

MSI patients. But there were also other histotypes, other than colorectal like cholangiocarcinoma and pancreatic and appendiceal cancer, so a variety of tumours. So, what is interesting is that also in this population, you have a high response rate, and this is in pre-treated patients. So it is of note that you have a high response rate in pre-treated patients and also, durable responses. Because there were partial responses and also complete responses in this patient population. And again also, the tolerability was as expected; mostly grade one and two and no new signals or worrying signals were reported in this analysis.

As for the other compound entrectinib, data were those presented last year at World Congress on Gastrointestinal Cancer, in GI tumours. Also, here we have a subset from a joint analysis of phase one and two data. In this case, we have data from 12 patients with *NTRK* positive GI tumours and also here, a high response rate, the durable responses have been noted in this cohort. Of course, as you know, as for all targeted therapies, the paramount problem for achieving long lasting results is secondary resistance, acquired resistance. And we know that this takes place under this therapeutic blockade mainly through on target mechanisms.

And of course, this is the main problem to overcome to obtain durable responses. Actually, we reported in 2016, the first case of a patient treated in my institution with *LMNA-NTRK* rearranged colorectal cancer with a secondary resistance. And in this case, indeed, a secondary mutation in the kinase domain of TRKA was observed. And interestingly, we monitored this resistant mutation also in the blood through liquid biopsy. Also, I think an interesting question to you Frédérique is from a biological point of view, based on this mechanism of resistance, what is the situation for these inhibitors in all cancers and also in GI tumours of course.

Frédérique Penault-Llorca
I think here we have, really the best indication for liquid biopsy, because it's a non-invasive, and we know that at least for *NTRK* most of the alteration are on target. And what I've been described so far, most of them are mutations in *NTRK* genes. So, this is really a very good way to monitor resistance, and to have a very early detection of a possibility of resistance. Of course, we all know the limitation of liquid biopsy. So, if it's negative and we truly have a clinical progression, we need to perform tissue biopsy to evaluate whether we have or not a mutation and maybe we can try to find for those patients if we have other therapeutic options for them to fight these resistance mutations.

Andrea Sartore-Bianchi
So, Frédérique based on what we said, what is your conclusions about *NTRK* fusion testing and treatment in GI tumours?

Frédérique Penault-Llorca
So, for me the take-home message is not to miss any patients. So, as we are now routinely performing MMR testing by immunohistochemistry, if we have dMMR profile, it's really easy to perform an immunohistochemistry test looking at the TRK proteins and if we have an overexpression, then we can look at the gene by NGS or by FISH. So, it would be my first message if we have a dMMR profile, and if we know that the patient is not KRAS and BRAF

mutated, we really need to reflex this immunohistochemistry. Andrea, what is your take-home message from the oncologist point of view?

Andrea Sartore-Bianchi
Yes, Frédérique, I think you said it is very important, and I agree, I very much agree not to miss any patients. I think that overall testing for *NTRK* fusions is an important opportunity in for example, in colorectal cancer because we have a broad molecular segmentation now, but these are all very limited subsets of patients. So, it's very important to push to find this patient for a therapeutic blockade that proved to be very effective, and also having this enrichment in the MSI-high, MLH1 hypermethylated subpopulation is also important because in this case, we have different therapies, we have also immunotherapy but again, we at least we know how to direct our diagnosis and also in this patient population it's a very important addition to the therapeutic armamentarium.

Frédérique Penault-Llorca
Andrea, thank you very much for this very lively discussion on *NTRK* fusion-positive GI cancers. And I hope all of you enjoyed our discussion as much as we did. Thank you so much.