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INTERVIEW

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“Neuroblastoma is a unique cancer in that it is more like a syndrome, representing a spectrum of similar diseases or tumors that all have quite different clinical behavior. This makes accurate diagnosis a very big priority...”

Neuroblastoma drug development: from lab bench to bedside?

Louis Chesler*

Louis Chesler speaks to Alice O’Hare, Commissioning Editor, about neuroblastoma clinical research.

Louis Chesler leads the pediatric solid tumor biology and therapeutics team at the Institute of Cancer Research (ICR; London, UK), one of the world’s most influential cancer research centers. Previously, Chesler worked for the US Government’s National Institute of Health and National Cancer Institute before joining the University of California, San Francisco (CA, USA) in 1995 to run a neuroblastoma research program and works as a paediatric oncology consultant. He moved to the ICR in 2007 as a senior clinical lecturer and is also an honorary consultant at the ICR’s partner hospital, the Royal Marsden NHS Foundation Trust. Chesler’s current interest is in developing new drugs for childrens’ cancers that at present respond poorly to existing treatment. These cancers include the three most common solid tumors of children: neuroblastoma, a nerve tumor; rhabdomyosarcoma, a muscle tumor; and medulloblastoma, a brain tumor. Part of Chesler’s work includes investigating the *MYCN* and *ALK* genes – which are abnormal in cancers derived from the brain and nervous system – and in developing novel therapeutics targeting these genes, an area of research currently attracting great interest. Chesler is a fellow of the Royal College of Pediatrics and Child Health, American Association for Cancer Research and the American Association of Pediatrics, and is active in several cooperative groups for development of novel cancer drugs, such as the Society of Paediatric Oncology European Neuroblastoma Network, Innovative Therapies for Children with Cancer Consortium, the new agents group of Cancer Research UK, and is on the editorial board for several peer-reviewed journals.

Q Can you please define what neuroblastoma is and how the condition is diagnosed?

Neuroblastoma is a tumor of developing nerve, and so most commonly we see it at diagnosis as a tumor in the area of the spine or adrenal gland (attached to the kidney), since tissues in these areas are derived from developing nerve. Since the kidney (adrenal gland) is the most common site of these tumors we often have to be careful to distinguish them from other primary kidney tumors of children. Usually this can be done easily with imaging, but we use genetic testing as well to help us diagnose the condition. Neuroblastoma was one of the first tumors where genetic testing, for the *MYCN* gene, took a very forward role in diagnosis and also in treatment, as it plays a very important role in determining survival and outcome. Frequently neuroblastoma is already metastatic by the time it is diagnosed, in children with high-risk disease, but there are many different presentations of this disease, from

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very curable and not metastatic, to very aggressive and difficult to cure.

Some of the factors that determine risk are dependent on the age of the child. So in very young children, we tend to see a more favorable outcome and the disease can look quite different in its presentation compared with, for instance, a child of 18 months or older, where there is the possibility for changes in the *MYCN* or other genes that increase risk, although this also is quite variable. Neuroblastoma is a unique cancer in that it is more like a syndrome, representing a spectrum of similar diseases or tumors that all have quite different clinical behavior. This makes accurate diagnosis a very big priority, since the way we classify neuroblastoma determines how we treat children, and we most definitely do not want to overtreat them, especially with conventional chemotherapy or radiation, which both carry toxicity and long-term side effects.

Q Following diagnosis of neuroblastoma, are there some general treatment regimens for the condition?

Yes, there are. In younger children who have more favorable or intermediate disease we generally use chemotherapy that is shorter in duration and less toxic, to avoid long-term side effects. However, what is unique about neuroblastoma is that it has the highest frequency in cancers of tumors that actually spontaneously disappear by themselves. Regressing cases usually occur in babies less than 6 months of age. This is again very unique, since some of those cases can actually have what looks like metastatic disease. This issue is really central to our understanding of the molecular wiring of this disease, because what it tells us is that this tumor is actually a disorder of developing nerve. So in the conditions where tumors regress spontaneously, what happens is that the ‘tumors’ seen at diagnosis may actually represent abnormal nerve tissue that is delayed in its development but it retains the ability to develop further, just not quite normally. Eventually these ‘tumors’ differentiate, or further develop into tissue that more closely resembles nerve, but doesn’t represent any cancer risk in the future. The issue critical to improved treatment of neuroblastoma is that if we could learn to stimulate this differentiation process in the very high-risk, difficult-to-treat tumors, we could cure more cases of neuroblastoma.

Other children have low-risk tumors without spread that can be treated with surgery or observation alone. In older children approximately a quarter of all patients have high-risk disease. We are still not quite sure what defines the majority of high-risk disease, but approximately half of patients have amplifications of the *MYCN* gene, which is an oncogene in neuroblastoma. Another

very important gene mutation discovered recently occurs in the *ALK* gene. We are continuing to discover more mutations that are associated with this disease that will help us to correctly classify the disease subtypes so that we can match patients with the appropriate treatments, and in the near future some of these will be novel small-molecules-targeted drugs that attack the protein products of these genes.

In high-risk disease though, the treatment is very difficult, and long-term survival is still less than 50%. The treatment unfortunately is very aggressive, involving combined multimodal therapy. This involves many cycles of combined chemotherapy agents, with surgery to remove any disease that does not respond, followed in some cases by external beam radiation and then by high-dose chemotherapy of the bone marrow transplant (termed ‘stem-cell rescue’). Then there is a final phase of treatment that uses a retinoid (such as *cis*-retinoic acid), which is a differentiating agent, and these days, the standard of care is to include an additional agent with a different mechanism of action. The most interesting development recently was the finding that the immunotherapeutic antibody to GD2, called CH14.18, is effective and increases long-term survival, when used with the immunomodulators IL2 and GM-CSF. Antibody therapy with other immune modifiers is now used as standard of care treatment for children with neuroblastoma in the USA, and there is an ongoing clinical trial of this approach throughout the UK and Europe to determine how to make this approach most effective.

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So to summarize, when a child has high-risk neuroblastoma this can be one of the most challenging tumors we encounter in pediatric oncology and necessitates maximal tolerated therapy, but with low-risk neuroblastoma only minimal or no therapy is required. All of this places huge emphasis on making a correct diagnosis as to the type of neuroblastoma a patient has. Our ability to classify patients correctly is very critical and will rapidly improve in the near future.

Q Does this new immunotherapeutic address some caveats in the present treatment options, or does it simply have an additive effect?

The problem with current treatment options available to us is that many of the classical chemotherapeutic agents we used have limited effectiveness and a lot of

toxicity. You can only use doses that are so high because of toxicity, and eventually, after repeated use, mutations develop in the tumors and the drugs lose effect. So a big challenge is to discover how to avoid repeated treatment in order to eliminate all traces of disease and prevent disease recurrence. We need to move to a model where we use targeted drugs that cause the tumor to regress to an undetectable state early on. Of course, that is a general challenge for most tumors and is a difficult problem to solve.

A very big problem in neuroblastoma is tumor recurrence, even in patients with no further detectable disease after standard treatment. In most high-risk patients we can usually get them into remission – and then we go on to give them all the other difficult therapies mentioned before. When you administer MRI and CT scans at the end of that treatment you can not see anything. However, we know that, without any visible X-ray or radiographic evidence of disease, or even these days molecular evidence of disease presence, many of our high-risk patients will go on to relapse.

So the addition of the retinoids and immunotherapeutics at the end of these long treatment protocols has the goal of eliminating the residual cells that we know hide in various parts of the body but are invisible. If we can improve our ability to target these cells (the minimal residual disease) our ability to extend the survival of patients will improve rapidly. That really is where one major current focus is in research and therapeutics.

The other current issue as mentioned, is that we don't really know the molecular definition of what high-risk disease is – we don't know how many genes are driving high-risk disease – and so it becomes hard to predict who will relapse and who will not. Therefore, we don't really have any personalized therapeutic approach so far and this is really what we need. This is a big focus in adult cancer research and we need a massive effort in pediatric cancer similar to this, if we really want to improve survival and reduce the damage we do to patients with toxic cancer drugs. This will obviously require a lot of research funding support, and we have to do a better job than we currently do in designing intelligent clinical trials of novel cancer drugs.

Q What research are you currently involved in within this research area?

A big problem in cancer research currently is understanding the function of cancer-causing genes in the laboratory and having valid ways of predicting whether a gene-targeted drug that is active in the laboratory will work in patients. The approach we take is to try and build preclinical systems and models that help us to

understand with some level of confidence or accuracy, whether the presence of mutations in targetable genes, such as *ALK* and *MYCN* – the two major mutations that occur in neuroblastoma – actually matters. We try to understand whether these genes can actually cause neuroblastoma on their own or whether they need to work with other genes to do so. If they do cause tumor formation, we find out how they signal, and if any of the pathways that are involved are targetable with new drugs. If no drugs exist, we have the capability at our center to make novel drugs. In this way we take a true 'bench-to-bedside' approach to deliver new drugs to the clinic.

Q Crizotinib recently showed positive results in a clinical trial with children suffering from neuroblastoma – can you describe the results of this study?

Yes, these exciting results were reported by Yael Mosse [1] who is a pediatric oncologist working at the Children's Hospital [Philadelphia, PA, USA], and who was one of the co-discoverers of *ALK* mutations in hereditary and sporadic cases of neuroblastoma. The trial ran through the Children's Oncology Group in the USA. The ability to conduct a trial like this in pediatric oncology reflects the input of many research groups and pediatric oncologists who worked to identify *ALK* mutations as being an important issue in neuroblastoma. *ALK* is the gene that is targeted by crizotinib and it plays an important role in hereditary cases of neuroblastoma and in sporadic disease. Regarding deficiencies in current treatment alternatives, we usually don't really know the molecular drivers that we can target in neuroblastoma. The more we are able to do this the better our treatments will be at preventing relapse and improving survival by using a new generation of molecularly targeted drugs. So the discovery of mutations in the *ALK* gene, which is mutated in approximately 10% of patients with neuroblastoma, and the availability of crizotinib, a drug that targets *ALK*, was really exciting. Crizotinib is among the first of a group of molecularly targeted therapeutics that we are using in pediatric cancer. These drugs actually attack a specific target gene, and therefore may avoid the toxicity of chemotherapeutic drugs that act more generally as toxins to cells that divide quickly. If we could completely define all pediatric cancers by their mutations and select cocktails of perfectly targeted drugs that inhibit them, in theory we could do a much better job of curing kids without toxicity. Since novel cancer drugs are not usually developed to primarily treat children's cancers, we were lucky that issues with *ALK* occur in a number of adult cancers, so that crizotinib had already been developed for these patients.

At ASCO in 2012 we heard that crizotinib was deliverable to high dose levels with generally minor toxicities, which were manageable, and although they didn't do upfront molecular testing of patients for *ALK* mutations, when they looked back, the drug was effective in many patients with these mutations. It was quite exciting that there were a number of complete treatment responses, which are rare in patients with heavily pre-treated, relapsed or refractory neuroblastoma, and also in anaplastic large-cell lymphoma (a type of lymphoma) and myofibroblastic tumors, other cancers where *ALK* mutations occur. So from this study the idea emerges, although not yet formally, that the activity of crizotinib correlates with *ALK* mutation state, and we may be able to make predictions about the future effectiveness of crizotinib from this study. Certainly this trial will lead to additional studies where crizotinib is used alone and with other agents, and also to studies of new *ALK*-targeted drugs.

Q In your opinion, what were the main findings of this study, and their implications?

The indication from the first trial is that it was a successful approach, that the drug may be effective in patients with abnormalities of *ALK* expression, and that you can deliver the drug safely at high doses. An important finding was that you can deliver higher doses in children than in adults. This matters because certain *ALK* mutations that occur in adult patients are resistant to crizotinib from the early clinical trials in adult oncology. In children, unlike adults, the mutations that occur in *ALK* are those most likely to be resistant to crizotinib, but these mutations seem to respond to higher doses of the drug. This is a nice finding because in this case we can communicate some data to the adult trials that may help to more clearly define how resistance to *ALK* mutations is mediated. This is a very good first trial that gives us excellent preliminary data on response in children with cancers that have abnormalities of *ALK* expression, and it gives us suggestions as to where subsequent trials should go. The most likely trial will be to genetically test patients for *ALK* at time of diagnosis and to combine crizotinib with chemotherapy to personalize their treatment, earlier in the treatment stratagem.

In addition, it suggests other future approaches, such as the use of second-generation improved *ALK* inhibitors, which are in development and have been reported in adult Phase I trials [2]. It also predicts that the use of antibodies to *ALK* may be effective, so we could take a similar immunotherapeutic approach like we did with GD2/ch14.18. This suggestion is based on the finding that a large proportion of patients actually express unmutated *ALK* on the cell surface in neuroblastoma

tumor but not normal tissue. This finding still needs thorough lab investigation, but if substantiated it would be very significant.

Q In a recent publication [3], you describe combining crizotinib with a second class of drugs – mTOR inhibitors – to knock out the resistance of cancer cells. In terms of clinical research, what implications does this research present?

Clinical resistance to crizotinib as a first-generation *ALK* inhibitor appears to be an emerging problem in adult patients who have *ALK* translocations, and there are some data showing that resistance may relate to secondary acquisition of *ALK* tyrosine kinase-domain mutants that have altered affinity for ATP. This is similar to the situation that occurs in patients with chronic myelogenous leukemia who are treated with Gleevec®. The primary mutation in an adult patient is that they overexpress fusion proteins of *ALK*, and are treated with crizotinib, but then become resistant because they develop point mutations in *ALK*. Primarily neuroblastoma patients have point mutations of *ALK* as the major mutation and so what we are trying to do next is circumvent the resistance associated with the treatment of these point mutations.

We know crizotinib is an excellent first drug but is not perfect, but we are trying to verify the clinical finding that if you use a higher dose level, it is more effective against these mutations. That is our primary strategy, to increase the dosage of this first-generation drug. The second strategy is to test novel, second-generation inhibitors that have been developed by drug companies, which may be more effective against these point mutations in *ALK*.

The third strategy is to combine drugs such as crizotinib or second-generation *ALK* inhibitors, with pathway-targeted agents where our laboratory research has told us that significant signaling in neuroblastoma occurs through these targetable pathways. The major targetable pathway we are investigating currently is the mTOR pathway. We are using specific kinase inhibitors of mTOR, because we know from our previous work, that the way *ALK* interacts with *MYCN* (the other important mutation in neuroblastoma) is through up-regulation of PI3-kinase mTOR pathway activity. So this predicts that if you use crizotinib with an mTOR kinase inhibitor, you should get dual activity – to treat both *ALK* and *MYCN* in neuroblastoma, and mutations in these two genes do co-segregate in high-risk patients. A major finding of the paper is that *ALK* signals the PI3 kinase mTOR pathway to stabilize *MYCN*. That is a major component of what *ALK* appears to do *in vivo*, and it gives us another therapeutic option

In addition, we know that some fraction of patients with an ultra high risk of neuroblastoma – who have *MYCN* gene amplification – frequently also express the F1174L mutation of *ALK* (those two co-segregate together). Therefore, a rational therapeutic approach is to target *ALK* with crizotinib or an improved *ALK* inhibitor, and add an mTOR kinase inhibitor that will target the PI3-kinase pathway, and the stabilization of *MYCN* by *ALK*. So, we will hopefully attack two important high-risk mutations simultaneously by doing that, and we hope this will also circumvent the resistance that may be a problem with crizotinib.

Q Do you foresee this preclinical research leading into clinical trials with patients?

We do. This is always a requirement of the research we do, which is by nature translational. We have future trial designs in mind when we conduct our ongoing research and the goal is to provide the laboratory-based evidence that clinicians need to propose these trials. The advantage of the *ALK* approach is that crizotinib was just tested in a clinical trial in adults and children, so we know it is safe. In addition, other mTOR inhibitors that have been developed, both here at the Institute of Cancer Research and at many pharmaceutical companies, have now been tested in adult patients. Therefore proposing a pediatric clinical trial, combining those two agents, in theory becomes much more practical. Whether or not these drugs have already been in adult human testing and are safe is a major prerequisite for proposing pediatric cancer trials. This is a persistent and important issue for us in pediatric oncology.

Q Are there other special considerations that need to be taken into account for pediatric clinical trials, in terms of trial design?

Yes, there are a number of very difficult issues in developing clinical trials in children's cancer. The first one is obvious. Thankfully, there are a small number of patients that have these tumors. This, however, makes it very challenging to plan the trials, because it takes a long time to conduct a trial and it makes interpretation of the clinical data difficult, because of statistical power.

Perhaps the greater challenge though is obtaining any novel drugs for use in pediatric cancer. The financial equation in pediatrics is very challenging for companies that invest the resources to develop them. If the patient population to be treated is very small, then the financial return, and investment for these very expensive drugs, is minimal. This is a huge challenge and we need another model that works for everyone, including, most importantly, the patients. We hope that our work

with major pharmaceutical companies will help them circumvent these issues. Maybe what we need to think about more is that if you cure a child with cancer, the benefit to society in the long run is huge – in years of life lost calculations, for example – in comparison to the resources we currently invest in treating adults with cancer. If we think that way, it helps us move most pediatric cancers to the top of any list that should receive funding for cancer research or drug development. Currently, things just aren't arranged this way, unfortunately. Obviously, the finances still don't work out for commercial drug development models, but they need to be looked at, since in adult cancer, essentially the problem that large companies are now facing is that most cancers are being redefined genetically into a large number of related entities that will all need specific targeted treatment. So the days of blockbuster drugs to treat huge numbers of patients are likely to be over, and what we may end up with eventually is a picture resembling the challenges we have always faced in pediatric cancer, although perhaps not to such an extreme extent.

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The third major challenge for pediatric trials is that the ethics of repeatedly obtaining tissue biopsies from children is not clearly accepted. In the current generation of molecularly targeted drug trials, the most important piece of information one could learn – or one requires – to interpret the effectiveness of a drug, is evidence of target modulation after treatment compared with pretreatment status. And generally, that data has to come from biopsied tumor tissue. Repeated biopsy of tumor tissue in children is uncommon, so our ability to learn about the effectiveness of new drugs is more complicated. We need to develop better ways to detect drug activity by imaging or other novel testing that isn't invasive or painful for children.

Q What model systems do you use in your research?

We mainly use genetically engineered murine models. We construct these models that then become useful preclinical systems for the research field to develop new drugs. But we are also using implantation models using biopsied human tumor tissue and are considering other more novel approaches for the future.

Q Looking at translating your research in transgenic murine models into human clinical trials,

what difficulties do you anticipate, and what challenges will need to be overcome?

There is a field-wide debate in translational drug development. The question is: what is the evidence that any preclinical, cell line or model system has predictive power to describe how a drug will behave in a patient on a clinical trial? The answer is that the data really do not exist at present. The transgenic models, and other approaches, we hope, will have a higher predictive ability and therefore will speed up the introduction of drugs into the clinic, with more predictable effects, however we have not yet achieved the goal in cancer research, although a lot of people are working very hard to solve this problem.

We hope the therapeutic strategies we have identified in our models will be successfully translated into clinical trials, because we think that our models are more representative of human tumors than some of the first-generation models or orthotopic systems. Because tumors in our models form spontaneously, they are immunocompetent and form in their native tissue of origin, with intact vasculature. So we think that makes the models much more useful.

Q What future work do you have planned in this field of research?

We have a remit to try to improve the survival of the difficult-to-treat pediatric solid tumors, which includes neuroblastoma, brain tumors such as medulloblastoma and glioblastoma, and muscle tumors such as rhabdomyosarcoma. In all of these areas, we are constructing these transgenic and other model systems to enable us to develop therapeutic approaches. The significant advance we are trying to make is to include these models in a ‘pre-clinical hospital’ that is efficiently set up or constructed to rapidly translate our preclinical findings into clinical use – hopefully with greater accuracy. We should be able to speed up the delivery of drugs to the clinic in this way.

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We are now working on new *ALK* inhibitors and new inhibitors of *MYCN*, since that gene is implicated in poor-outcome subgroups of patients that have all of those conditions I just mentioned. We are also trying to develop rational combinations of chemotherapeutic agents, plus targeted agents, which will enable us to reduce the doses used of standard chemotherapeutics.

Q What do you believe the future of neuroblastoma treatment holds?

I fully anticipate – or believe – that if we can use these models to understand well enough how these tumors initially develop, at very early time points, before they have acquired enough mutations, or genetic complexity to make them untreatable, that were we to find safe and nontoxic agents – that we could use very early – we should be able to cure some pediatric cancers prior to their development through these approaches.

This is, of course, all very complicated. You have to theoretically develop a clinical test with very high predictive capacity, very high sensitivity and specificity, to tell you that one of these tumors is going to have either an excellent or poor outcome – so you have a way to detect them very early and with great accuracy and predictive power. For the approach to be ethical, you also have to have a safe enough drug to use, so that the benefit matches the risk to the particular child. This presents clear ethical issues. However, theoretically, we think we should be able to identify safe and active enough agents that, in combination with the appropriate clinical tests, we should be able to move to a new model for treatment which promises safe cures for some of these cancers.

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