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BLADDER CANCER, WHEN BASIC AND CLINICAL RESEARCH MEET

It is both an honor and a pleasure for me to write about bladder cancer research on the occasion of the 70th birthday of Professor Popov, my friend Zivko.

I have always enjoyed meeting up with Zivko, sharing his passion and enthusiasm, and watching the expression on his face switch between facetious and extremely serious. It has never ceased to amaze me how such a skilled surgeon also manages to display such dedication to his research projects. I have been lucky enough to have the opportunity to work with him, both while he was working at Henri Mondor Hospital in Créteil, and in Skopje, in the framework of a European project tackling a difficult question: why some non-muscle-invasive bladders recur, whereas others do not.

It is no overestimate to say that Zivko's research work with Dominique Chopin in France (Chopin *et al*., 1993; Mazerolles *et al*., 1994; Popov *et al*. 1997, 2000, 2004; Saint *et al*., 2004) shaped my career. Zivko and Dominique, whose names and contributions are often associated in my mind, were among the first to understand the importance of annotated tumor banks and the key role of surgeons in research studies based on this invaluable resource. They were also well ahead of the curve in understandding the value of multidisciplinary collaborations in the field of cancer research. I learnt a lot from may interactions with Zivko, Dominique and the young urologists training with them at the time at Henri Mondor Hospital, who were attracted by the possibility of performing research in an environment in which laboratory and clinic were closely linked: Marc Colombel,

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Jean-Jacques Patard, Vincent Ravery, and Fabien Saint, to name but a few. I also remember Sixtina Gil Diez de Medina, an MD who trained in Chile before joining the team at Henri Mondor, as an essential element of this collaborative atmosphere. Through these interactions, I got to know many influential MDs from France and elsewhere including, in particular, two pathologists: Yves Allory, a long-standing collaborator of mine who is now at Institut Curie, and Theo van der Kwast, who was working in Rotterdam at the time and is now in Toronto. Unfortunately, Dominique is no longer with us, but he will always remain in our memories.

Zivko, the time I spent in Skopje, at your invitation, will remain with me forever as an unforgettable moment of science, culture and friendship. I wish we could have worked more together, and had more opportunities to meet.

We have witnessed the progress in both basic and clinical aspects of bladder cancer research, and have, on occasions, contributed to it. This progress was made possible by the persistence of several teams, including several teams in Europe, and by technical advances, particularly in molecular biology and data analyses. The success of recent clinical trials of immune-therapies and targeted therapies has added a further impetus to this progress.

Many technical advances have been made in the last few years, driving considerable progress in our molecular understanding of normal and pathological processes, including cancer. This technical revolution began with the sequencing of the human genome. The advent of microarray technology was the first major step forward in this process, making it possible to study the expression of thousands of genes simultaneously. This technology was then applied to studies of the genomic alterations occurring in cancer. A few years later, high-throughput sequencing was developed. This technology revolutionized sequencing, by greatly decreasing its cost, and making it possible to identify polymorphisms (the differences between two individuals at the DNA level), somatic mutations (mutations occurring during tumorigenesis), copy number alterations, and translocations. Not only was high-throughput sequencing used to study the genetics of cancer, but it was also used to investigate changes at the epigenetic level (the epigenome is all the modifications making two cells with identical genomes different). High-throughput sequencing can be used to study the DNA methylome, but also the proteins bound to DNA and their chemical modifications, DNA accessi-

bility and the three-dimensional structure of the DNA (two regions of DNA, even if separated by large distances, can interact, through the creation of loops). The revolution continues, and it is now possible to perform single-cell studies of many parameters that just a few years ago could only be studied on thousands of cells at a time. Transcriptomes, genomic alterations, methylomes, histone modifications, and DNA accessibility can be now studied in single cells, and such studies have revealed an unsuspected level of heterogeneity in tumors. Single-cell analyses are generally performed on cells obtained by tissue digestion, resulting in a loss of spatial information. Such information is crucial to place the cell in its context, and to identify its neighbors and possible mechanisms of cell-to-cell communication. Fortunately, *in situ* techniques have progressed at the same pace as techniques for precisely localizing proteins (by multiplex immunofluorescence) and RNA (spatial transcriptomics, named technology of the year in 2020 by the journal Nature). With the resulting data avalanche, data analysis has become crucial, and bioinformaticians and statisticians have become essential actors in biology generally, and in cancer research in particular, resulting in an ever-growing need for multidisciplinarity in research.

I would like to take the opportunity here to introduce our work on FGFR3, an Ariadne's thread of a research adventure building on the foundations laid at Henri Mondor Hospital. FGFR3 is now recognized as a major actor in bladder cancer. It is activated by point mutations in about 50% of non-muscle-invasive bladder cancers (NMIBC) and 15% of muscle-invasive bladder cancers (MIBC) (Cappellen *et al*., 1999; Billerey *et al*., 2001; Neuzillet *et al*., 2012). In rarer cases (about 4% of MIBC), it may also be activated by fusion (Williams *et al*., 2013). FGFR3 is a tyrosine kinase receptor, and is therefore a possible therapeutic target. Indeed, the favorable results obtained in recent clinical trials (Loriot *et al*., 2019) have led to FDA approval for anti-FGFR therapy in patients with advanced bladder cancer with tumors presenting *FGFR3* mutation or fusions of either FGFR2 or FGFR3 (*FGFR3* mutations are the commonest of these three events, occurring in 75% of cases).

The first identification of *FGFR3* mutations (Cappellen *et al*., 1999) by my team (then part of Jean Paul Thiery's laboratory), in collaboration with Henri Mondor Hospital, was only possible because of the bank of frozen tumors set up by Dominique and Zivko. This bank was highly

diverse, containing bladder tumors of various stages and grades, and it was entirely unique at the time. We are still using it today, more than thirty years after its establishment. The protocols we used to obtain not only DNA, but also RNA and proteins, from the tumors in this tumor bank were robust. We took our time at the start, carefully checking the quality of the protocol initially described by the group of M. Knowles (Coombs *et al.,* 1990), but it paid off. The RNA and DNA samples we obtained remain undegraded and can still be used for all the techniques our research demands. We initially performed targeted studies on a few genes/proteins, such as FGFs and FGFRs, then large-scale DNA and RNA analyses (Stransky *et al*., 2006; Biton *et al*., 2014; Rebouissou *et al*., 2014), and we are now performing large-scale analyses on proteins (Sanchez-Quiles *et al*., 2021). The techniques are evolving and the tumor bank is still going strong.

Before FGFR3, FGFR2

Why did we start to work on FGFR3, one of the four members of the FGFR tyrosine kinase receptor family? When we started this work, large-scale mutations analyses had not yet been developed, so we had to focus on a small number of genes. The team at Henri Mondor Hospital was interested in FGFs, the ligands of FGFRs, because these factors could be found in the urine. For this reason, we focused on the FGF receptors in bladder tumors, although, with hindsight, we have still not found the link between the important role of FGFR3 in bladder cancer and the presence of FGFs in urine. The first FGFR we studied was FGFR2. The main isoform of this receptor expressed in the bladder urothelium (the tissue of origin of most bladder cancers) is FGFR2b. We unexpectedly found that FGFR2b was lost during tumor progression and that it inhibited the growth of bladder tumor cells. So, FGFR2b, which, as a tyrosine kinase receptor, we had expected to play a protumorigenic role, actually had the opposite effect in bladder tumors (Gil Diez de Medina *et al*., 1997, 1999; RIcol *et al*., 1999). Many examples of proteins that play opposite, inhibitory or protumorigenic roles in different tissues and differentiation states are now known. FGFR3 is another striking example. It inhibits bone growth (activating mutations of *FGFR3* in the germline are responsible for several forms of mild to severe dwarfism), and has a protumorigenic role in various tumors.

Activating *FGFR3* mutations in bladder tumors

Our results for FGFR2 were not what we had expected. We were searching for tyrosine kinase receptors with a tumorigenic role to serve as potential therapeutic targets. We therefore moved on to another FGFR expressed in the urothelium: FGFR3. We initially feared that history would repeat itself and, indeed, we observed a downregulation of FGFR3 in a significant proportion of bladder tumors and several other indications of a possible tumor suppressor role for FGFR3. We therefore sequenced *FGFR3*, looking for inactivating mutations. To our surprise, we found a large number of recurrent mutations (Cappellen *et al*., 1999), all of which had already been reported as germline mutations in a severe form of dwarfism, thanatophoric dysplasia (Tavormina *et al*., 1995; Rousseau *et al*., 1995). These mutations had already been shown to be activating mutations (Naski *et al*., 1996), providing genetic evidence that FGFR3 can act as an oncogene in bladder cancers. We then joined forces with the team of Ellen Zwarthoff and Theo van der Kwast. Theo was already collaborating with the team at Henri Mondor Hospital, and we performed a systematic search for *FGFR3* mutations in bladder cancer. Remarkably, most of the mutations we found were mutations already implicated in thanatophoric dysplasia, but other rarer mutations were picked up that had also already been reported as germline mutations in other forms of dwarfism or bone diseases (achondroplasia, hypochondroplasia, Crouzon syndrome with acanthosis nigricans) (van Rhijn *et al*. 2002).

Funnily enough, David Cappellen — the student in the team working on FGFR2 and FGFR3, who brought the techniques he had learnt at the Gustave Roussy Institute that were essential for the identification of *FGFR3* mutations to the laboratory — described only the possible tumor suppressor role of FGFR3 in his PhD thesis. We discovered the activating mutations just after he submitted his thesis. We had also already submitted a manuscript in which we described the inhibitory role of FGFR3. I called the editor to explain that we now had genetic evidence of an oncogenic role for this receptor. To my surprise, the editor was immediately convinced, willing to wait for the modifications, and he then sent the manuscript for review. It turns out that FGFR3 in the bladder urothelium, may have either an oncogenic role or a tumor suppressor role, and the story of its dual role does not end here. We filed a patent for the identification of *FGFR3* activating

mutations, as this discovery revealed that FGFR3 could be used as a therapeutic target and diagnostic marker for certain types of bladder cancer. Institut Curie helped us with the patent application, but we also had help from another David, David Ricol, a former student from the team who had worked on FGFR2 and FGFR3 during his PhD before studying to become a patent attorney. This patent was contested by Genentech and Roche, which annoyed me considerably, but David Ricol told me not to worry, it was a good sign, indicating that they were interested. Genentech and Roche got hold David Cappellen's PhD thesis, in an attempt to show that we had disclosed the information before the patent application was filed, which would have invalidated the patent. However, as the activating mutations were not discovered until after the submission of this thesis manuscript, the oncogenic role of FGFR3 was not in it and only the tumor suppressor role of the receptor was described!

Inverse correlations of FGFR3 mutations with stage and grade

Thanks to the annotations of the Henri Mondor tumor bank (pathological and clinical data were available for all the tumor samples) and the involvement of a young pathologist, Marie-Hélène Aubriot-Lorton, whose Masters project focused on FGFR3, we were able to detect an inverse correlation between the *FGFR3* mutation frequency on the one hand, and grade and stage on the other. The percentage of *FGFR3* mutations was high in G1, lower in G2, and very low in G3 tumors (including carcinoma *in situ*, which is always of high grade). A similar pattern was observed for stage, with the percentage of *FGFR3* mutations high in Ta tumors (papillary tumors not invading the basement membrane), lower in T1 tumors (invading the basement membrane but sparing the smooth muscle) and even lower in T2-4 (tumors invading the smooth muscle). Discussions with two pathologists specializing in bladder cancer, Claude Billerey and Dominique Vieillefond, suggested that the inverse relationship with stage might be even stronger, due to the possible misclassification of some Ta as T1. Dominique Chopin and the Head of the Pathology Department at Henri Mondor Hospital, Serge Zafrani, agreed that Claude Billerey and a pathologist from the Department, Marie-Pierre Bralet, should re-examine the slides. This review

of the slides, blind to *FGFR3* mutation results, indeed revealed that several of the mutated T1 tumors were actually Ta tumors.

This inverse correlation between *FGFR3* mutations and stage or grade (Billerey *et al*., 2001) was initially puzzling. However, it turned out to be easily explained by the existence of two pathways of tumor progression in bladder cancer: the low-grade Ta pathway and the CIS pathway, consistent with the initial observations of clinicians (progression is rarely observed for low-grade Ta tumors, but frequently observed for carcinoma *in situ* (CIS), high-grade tumors that do not invade the basement membrane. *FGFR3* is a driver gene for the Ta pathway, but not for the CIS pathway. The percentages of *FGFR3* mutations found were completely consistent with this hypothesis: 70% in TaG1 and TaG2 tumors, none in CIS, and 15% in T2-4 tumors (about 80% of MIBC arise from CIS). An association had previously been found between *TP53* mutation and the CIS pathway. *TP53* mutations are absent from Ta low-grade tumors, but are frequent in CIS and MIBC (Spruck *et al*., 1994). It is essential to think in terms of the existence of different pathways. Not doing so would results in the conclusion that FGFR3 is initially important for tumorigenesis but not after progression. This conclusion is entirely incorrect. Tumors with *FGFR3* mutations continue to express FGFR3 when they progress and continue to be dependent on this receptor. This situation is reminiscent of that in chronic myeloid leukemia, in which the progression of the tumor is built around the activation of an oncogene, BCR-ABL.

Take care when sampling*:* *FGFR3* may also be mutated in cervical cancer, albeit rarely.

After discovering *FGFR3* mutations in bladder cancer, we decided to check for the presence of these mutations in other carcinomas. We were already working on cervical cancers in collaboration with Xavier Sastre-Garau, Head of the Pathology Department at Institut Curie. This seemed like a good place to start, so we measured FGFR3 expression in cervical cancers. Expression levels ranged from absent to very high. We selected 12 tumors with different levels of FGFR3 expression and sequenced them to check for

FGFR3 mutations. We found activating mutations in three of these tumors, which led us to conclude that *FGFR3* mutations were also frequent in cervical cancer. We subsequently realized that *FGFR3* mutations were less frequent in cervical than in bladder cancers (Rosty *et al*., 2005). Our initial observation was biased due to the small number of cervical tumors studied and the non-random nature of their selection (based on FGFR3 expression). All the mutations were found in cervical tumors with high levels of FGFR3 expression (as for bladder cancers).

Functional evidence for an oncogenic role of FGFR3 *in vitro*

Activating mutations provided genetic evidence for a protumorigenic role of FGFR3 in bladder cancer. We then looked for functional evidence of this oncogenic role. Whilst visiting Yves Fradet's laboratory in Canada (we were introduced by Dominique Chopin), I was informed that they had a a cell line derived from NMIBC — the MGHU3 cell line. This cell line was therefore possibly mutated for FGFR3. They sent it to us. we checked for and found activating mutations of *FGFR3* in this cell line, which we were then able to use to investigate the functional role of FGFR3 in bladder cancer. Fortunately, the arrival of this cell line coincided with the arrival in the laboratory of a young researcher, Isabelle Bernard-Pierrot, who was highly motivated by this project and had expertise in functional studies. We showed that FGFR3 inactivation by siRNA approaches or with an FGFR inhibitor in this cell line led to a loss of its tumor properties. We also showed that mutated *FGFR3* could transform an immortalized cell line (Bernard-Pierrot *et al*., 2006), thus demonstrating a tumorigenic role for mutated FGFR3 *in vitro*.

Functional evidence for an oncogenic role of FGFR3 *in vivo*: from bladder tumors to benign skin tumors

We investigated the possible tumorigenic role of FGFR3 *in vivo*, by generating transgenic mice expressing mutated *FGFR3*. We studied the most common mutation of *FGFR3*, FGFR3 S249C. As our goal was to demonstrate the possible oncogenic role of FGFR3 in the bladder urothelium in

particular, but possibly more generally in epithelia, we used different promoters to target the expression of the mutated *FGFR3* to the urothelium (the promoter of the uroplakin 2 gene, which I obtained after my visit to Tung-Tien Sun's laboratory in New York), and to other epithelia (the promoter of the keratin 5 gene, obtained from Jose Jocarno in Madrid). We derived several lines for each construct. Our first striking observation concerned the keratin 5 promoter – FGFR3 S249C-transgenic mice. They had verrucous lesions on the snout and eyelids, and older mice also had skin lesions on the throat and upper chest. Histologically, these lesions resembled benign skin tumors. In parallel, we investigated *FGFR3* mutations in a panel of various non-bladder carcinomas, including skin carcinomas (squamous cell carcinoma and basal cell carcinoma); we found no *FGFR3* mutations in these lesions (Karoui *et al*., 2001). In addition to observing these skin lesions in keratin 5 promoter – FGFR3 S249C mice, we also performed laser microdissection on various epithelia, and we found that FGFR3 was strongly expressed in the urothelium and epidermis. We therefore expected to observe frequent *FGFR3* mutations in skin cancers, as in bladder cancer. During a discussion of these results at one of our laboratory meetings, a pathologist, Christophe Rosty, said that the skin lesions in the transgenic mice resembled benign skin tumors observed in humans and suggested that we look at *FGFR3* mutations in the most common benign tumor in humans, seborrheic keratosis. The tumor bank at Institut Curie included several such lesions, so we rapidly investigated *FGFR3* mutations in these benign tumors. We identified the very same *FGFR3* mutations that we had found in bladder cancer and had previously been identified in patients with thanatophoric dysplasia at a high frequency in these lesions (Logié *et al*., 2005). Thus, activating mutations of *FGFR3* inhibit growth in bone, they induce proliferation but never transformation in the epidermis, and they cause proliferation and transformation leading to low-grade tumors in the urothelium that may, in rare cases, progress to MIBC. The reasons for these differences remain unclear. We found that even though the *FGFR3* mutations observed in bladder cancer and seborrheic keratoses were the same, their distributions were different, with FGFR3 S249C the main mutation in bladder cancer, whereas this mutation was no more frequent than other *FGFR3* mutations in seborrheic keratoses. At the time, we interpreted this observation incorrectly as indicating that FGFR3 S249C was more transforming than other *FGFR3*

mutations. We performed a number of observations to test this hypothesis, but were eventually forced to conclude that the transforming ability of FGFR3 S249C was no higher than that of another mutation we tested, Y375C.

We also generated transgenic mice expressing mutated *FGFR3* under the control of the uroplakin 2 promoter. Initial observations revealed hyperplasia of the urothelium in these mice (this model is more difficult to study than keratin 5 promoter – FGFR3 S249C mice, in which the tumors are on the skin and their appearance is easy to follow). Isabelle Bernard-Pierrot had the patience to follow a large enough number of mice for sufficiently long periods of time to observe lesions, which Yves Allory and Jacqueline Fontugne, a pathologist who had joined his team, identified as low-grade Ta tumors. The expression of mutated *FGFR3* in mouse urothelium therefore reproduced the very frequent association of *FGFR3* mutation with low-grade Ta tumors observed in humans. A bias toward higher tumor penetrance in males was observed in the mouse model. We checked the human data and found that the same was true for humans: the male-to-female ratio was higher in patients with *FGFR3*-mutated tumors than in patients with non-mutated tumors, suggesting a role for the androgen receptor in the process of carcinogenesis in tumors with *FGFR3* mutations.

An explanation for the higher frequency of FGFR3 S249C in certain cancers, including bladder cancer

There are observations for which the explanations remain elusive for long periods, suddenly crystalizing years later. In response to an invitation from David McConkey, I once gave a seminar at Johns Hopkins University focusing on FGFR3, in which I suggested that the high frequency of the FGFR3 S249C mutation was due to a greater transforming activity of this mutation. At the time, I was 100% convinced by this theory. The following day, I went to Bethesda/Rockville to visit several other scientists, including, in particular, Mila Prokunina-Olsson at the NIH, who was working on polymorphisms associated with bladder cancer, which was a subject of great interest to our laboratory, as it can help to identify genes of importance in bladder carcinogenesis. Whilst preparing for my meeting with her, I was in my hotel room reading one of her papers on a polymorphism associated

with a gene coding encoding one of the APOBEC enzymes (a large proportion of the mutations in bladder cancer are due to APOBECs). Looking at the sequence of the motif recognized by APOBEC, it suddenly hit me that the higher frequency of FGFR3 S249C could not be due to an advantage of this mutation over other *FGFR3* mutations, but to the mutagenesis process, which preferentially targeted the S249 site. I checked and found that S249 was the only *FGFR3* mutation with an APOBEC motif. At this point, all this was just an idea, but I discussed this idea with Mila and then with the members of my laboratory on my return. Having an idea is just the first step. Many ideas remain just that if they are not realized, choosing the right idea and demonstrating is validity, is probably the most important step. Two very motivated students in the laboratory, Mingjun Shi and XIangYu Meng, were immediately convinced by this idea, as was Isabelle Bernard-Pierrot. They interrupted the studies they were working on, and investigated it. If FGFR3 S249C was due to APOBEC, then APOBEC signatures should be observed in tumors carrying the FGFR3 S249C mutation than in tumors carrying other *FGFR3* mutations. A modest but significant correlation was observed in the TCGA data, but these data include only MIBC tumors, relatively few of which have *FGFR3* mutations. We wrote to a Danish researcher, Lars Dyrskjøt (I was a member of his PhD panel, and he now heads a very active group working on bladder cancer). Lars had molecular data for a large series of NMIBC tumors, thanks once again to the existence of a large tumor bank set up over a number of years and initiated by his former boss, Torben Orntoft. The frequency of *FGFR3*-mutated tumors was high in this tumor bank, and he found a highly significant correlation between FGFR3 S249C mutation and the APOBEC mutational signature. This and other observations in other tumor types with and without a higher frequency of FGFR3 S249C mutation than of other *FGFR3* mutations led us to conclude that the carcinogenesis process may be biased toward particular recurrent mutations. These recurrent mutations could be driver mutations, like FGFR3 S249C (Shi *et al*., 2019) or even passenger mutations (Shi *et al*., 2020).

FGFR3 mutation and molecular subtypes of MIBC

Bladder cancer is a very heterogeneous disease, both clinically and histologically. Many attempts have been made to identify molecularly

homogeneous classes of tumors that could be used to predict the outcome for a given patient and the response to treatment. One of the first cancers for which such a strategy, based on transcriptome data, was applied was breast cancer (Perou *et al*., 2000; Sorlie *et al*., 2001). Several subtypes, including the basal and luminal subtypes, were identified. Basal tumors express genes that are also expressed by the cells of the basal layer of stratified epithelia, and have low levels of differentiation markers. Luminal tumors express the estrogen receptor and differentiation markers. Luminal breast tumors are usually hormone-dependent, whereas basal tumors are not. We and other teams have also used transcriptomic data to identify homogeneous subtypes of bladder cancer (for example, Cancer Genome Atlas Research Network 2014; Hurst *et al*., 2017; Robertson *et al*., 2017; Marzouka *et al*., 2019; Kamoun *et al*., 2020; Lindskrog *et al*., 2021). In these studies, it was important to consider NMIBC and MIBC separately, because these two groups of tumors constitute different diseases in terms of their clinical features. Separating them improves the granularity of each category. Strikingly, in all these studies, attempts to identify molecularly homogeneous classes identified a main division separating the tumors into two groups: basal (also called basal/squamous) and luminal tumors. As for breast cancer, basal bladder tumors express markers of the basal layer of stratified epithelia and have low levels of differentiation markers, whereas luminal bladder tumors express high level of differentiation markers. The similarities between bladder and breast cancers extend even further: both basal bladder cancers and basal breast cancers are particularly aggressive, with deaths occurring earlier than for luminal cancers; furthermore, luminal bladder cancers are also dependent on a nuclear receptor, not the estrogen receptor as for luminal breast cancers, but PPARγ (Biton *et al*., 2014; Rochel *et al*., 2019). The luminal subtypes of MIBC include one particular subtype identified by several teams: the luminal papillary subtype (described as “papillary” on the basis of the frequent papillary morphology of these tumors). Most of the *FGFR3* mutations are found in tumors of this subtype (2/3 of tumors with *FGFR3* mutations belong to this subtype, and 40% of the tumors of this subtype present mutations of this receptor gene). In addition, all the tumors of this subtype (not just those with *FGFR3* mutations) present a transcriptomic signature of activation for this receptor. Several questions have been posed: do the luminal papillary subtype tumors without *FGFR3* muta-

tions respond to anti-FGFR therapy? Does the response to anti-FGFR3 therapy differ between tumors with *FGFR3* mutations of the luminal papillary subtype and other subtypes? Given that the stroma of the luminal papillary tumors is particularly poor (small numbers of normal cells in the tumor microenvironment), it also remains unclear whether the response to immunotherapy or combination therapy (anti-FGFR and hormonotherapy) is likely to differ between patients with luminal papillary tumors with *FGFR3* mutations and patients with mutated tumors of other subtypes.

Dividing tumors into molecular categories (not necessarily based on the transcriptome) may be useful not only for predicting treatment response, but also for identifying prognostic markers. For example, in studies considering only NMIBC tumors with *FGFR3* mutations, we identified a marker of progression: *CDKN2A* loss. This finding was based on the observation that mutated MIBC tumors often display CDKN2A loss, whereas mutated NMIBC tumors do not, suggesting that *CDKN2A* loss is an important event in *FGFR3*-mutated tumors (Rebouissou *et al*., 2012).

The mutated FGFR3 signaling pathway: the adventure continues

Not all patients with *FGFR3*-mutated MIBC respond to anti-FGFR3 therapy, and initial responders eventually relapse. It is, therefore, important to identify the patients likely to present an initial response to treatment, and to develop additional treatments. Studies of the signaling pathway of mutated FGFR3 could potentially lead to the identification of additional therapeutic targets. Isabelle Bernard-Pierrot has tackled this problem, successfully identifying important actors in this signaling pathway. In particular, she identified a positive feedback loop between mutated FGFR3 or fusion proteins involving FGFR3 and the transcription factor MYC. Mutated FGFR3 and FGFR3 fusion proteins activate MYC, in turn activating FGFR3 expression. She has also begun to identify intermediate proteins involved in this loop that could potentially serve as therapeutic targets (Mahé *et al*., 2018). One key aspect of resistance to treatment is heterogeneity (the existence of several types of tumor cell, differing genetically and/or epigenetically, within a tumor), and another is plasticity (tumor cells can change their phenotype in response to the tumor microenvironment and treatment). Heterogeneity and

plasticity are mediated by changes in signaling pathways and the activation of transcription factors. Host polymorphisms and, probably, the history of patients (the environmental factors to which patients have been exposed) may also be important for predicting the response to treatment. Many exciting discoveries of benefit to patients are undoubtedly yet to come.

I must thank Zivko and Dominique for their enthusiasm for bladder cancer research, for attracting me into this field, for introducing me to talented researchers and MDs, for having understood the importance of annotated tumor banks before it became widely apparent, for teaching me about the clinical aspects of this disease and for demonstrating the importance of multidisciplinarity.

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