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A Sophism in Vectorology: Turning Harmful Defective Retroviral Vectors into Helpful Replication-Competent Retroviruses Against Cancer

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“As each thing appears to me, so it is for me, and as each thing appears to you, so it is for you.” Protagoras (as cited in Plato’s *Cratylus*, 386a)

“Of all things the measure is Man, of the things that are, that they are, and of things that are not, that they are not.” Protagoras

I bumped into a smiling guy as we headed to the same spot, the far end of a large poster hall of the Third Annual American Society of Gene Therapy (ASGT) Meeting in 1999 in Washington DC. It was as if we had been banished to the remotest part of the hall, so as not to be seen. We unfolded and hung our posters. And we understood. Nori Kasahara and I were both presenting our results on the development of replication-competent retroviral (RCR) vectors for gene therapy (of cancer). And this was right at a time when everyone who wanted to use defective retroviral vector for a clinical trial knew they would be scrutinized by the regulatory agencies for the presence of RCR in their vectors’ preparations. Any RCR and the preparation were trashed. And there were scientists seriously thinking of using RCRs for gene therapy! How is that possible?

For me, it all started because of the results of our clinical trials of suicide gene transfer for the treatment of patients with glioblastoma (GBM)¹ and melanoma.² Preclinical results on the use of suicide genes to treat experimental cancer in rodents had been spectacular.^{3,4} The experimental system appeared to be loaded with fantastic properties. Killing cells was clearly an easier task than fixing them, as needed for the treatment of genetic

diseases. There were no (less) issues on how much transgene expression was needed, and for how long. Killing cells with suicide genes, which needed the combined expression of the gene and the administration of a prodrug (ganciclovir [GCV] in our case), appeared to have robust safety features. Indeed, the retroviral infection required cell division and so did GCV’s killing mode of action, together ensuring good specificity of the killing of tumor cells. And there were these fantastic bystander effects—local⁵ and distant^{6,7}—which alleviated the need for very efficient gene transfer (or so we thought). So, after seeing these large liver tumors disappear in rats,⁴ I enthusiastically turned to the translation to clinical evaluation, which proved to be a bumpy road.

At the time, scientists (at least me) hardly knew what “good manufacturing practices” (GMP) meant. I rapidly learned two things about them: they are labor-intensive and expensive. So, we now had to look for additional funding. In France, at the time, there was no specific funding for translational research in gene therapy, only for experimental preclinical research. Requests for significant amounts of money just to produce a “clean” packaging cell line were received by grant committees with comments such as “this is not science.” To cut a long story short, after trying GMP production with the only two companies “capable” of producing retroviral vectors in Europe at the time, and after depressing failures, we had no choice but to do it ourselves—which meant launching, with my colleague and friend Jean-Loup Salzmann, a biotech

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company that we pompously called Génopoiétique (what a nice name!). Start-ups were another discovery, plus working with other types of people. Génopoiétique's first goal was to set up the GMP production of retroviral vectors, with the help of Rhône-Poulenc Rorer (now Sanofi). Thanks to the talent of the late André Crespo, it worked, and in a timely fashion, we produced our first approved batches. Incidentally (and ironically), at the same time, we produced the first batches of retroviral vectors used for the first trial of gene therapy of severe combined immunodeficiency,⁸ the very trial that raised concern about the safety of defective retroviral vectors...

With our vectors, we launched what were at the time the first two European cancer gene therapy clinical trials, based fully on local research, development, and production. This was at the time when *Human Gene Therapy* had started to publish clinical trials.^{1,2} These trials were the first to go through the regulatory committees that had just been formed at the time in France, under the direction of Jean-Hugues Trouvin. Having had to learn about, and then deal with, GMP production, we now had to learn about regulatory issues and in fact help to build with the committee what became the standards for the upcoming trials. We finally received regulatory clearance for our trials.

The treatment modality was to inject the packaging cells producing the defective retroviral particles that transduce suicide genes straight into the tumor. Compared to the injection of viral particles, we thought that injection of packaging cells would improve tumor cell transduction. GBM and melanoma were chosen for reasons that would take too long to explain here. They retrospectively have been worst rather than best choices. In these first-in-man trials, we had to treat advanced (euphemism) diseases. We could not treat all the multiple lesions of our melanoma patients, and although we saw remarkable regression of some injected tumors, the distant bystander effects were obviously insufficient to take care of the enormous disseminated tumor mass from which these patients suffered.⁹ For the GBM patients, the setting looked more favorable, with a tumor that often appeared to be well limited on magnetic resonance imaging (MRI). Patients had their tumor surgically debulked, and the cells were injected quite cautiously and meticulously all around the tumor margin. The atmosphere in the operating room was quite something, with an entire team of nurses and doctors having the impression that they were making history. We treated 12 patients, with "mixed" results.¹⁰ At 4 months after treatment, four patients

had no recurrence, and later had an overall survival almost three times longer than patients with recurrence. One patient was still free of detectable recurrence, steroid free, and independent 2.8 years after treatment, which was quite unusual. So, the glass looked pretty empty to most of us. However, one of most striking observations for me came from the postmortem pathological studies. It is the tradition in my hospital that the brains of patients are macroscopically examined in the presence of the entire staff of doctors and students, in a "Charcot style session." The entire brain is placed on a plate, and is then sliced. Having skipped neuropathology during my medical studies, the vision was quite disturbing. These were the brains of people I had been speaking with just a few days earlier—people who had placed in us their last hopes, and whom we had failed. However, these sessions revealed something that puzzled me greatly. Even on macroscopic slices, it was so obvious that the tumor was not the well-limited mass we saw on the MRI. There were migrating tumor cells everywhere, even in the contralateral hemisphere. I knew from the literature that GBM does not generate metastases but is disseminated locally. But to that extent? How naïve I had been to think that we could target these cells with our technique.

This could have been it: empty glass, failure, end of the program. But the enormous amount of work—of team work—put into this research and my optimistic nature made me see something in the glass. It was not even half-full, but something was there. The one long-term survivor at the time that we wrote our report later died from disseminated breast cancer, with still no indication of tumor recurrence on MRI. Postmortem analysis confirmed that there was no evidence of recurrence near the initial tumor localization or in any other area of the brain. The pathologists were amazed and agreed to conclude a case report with "Such an evolution is unusual in the course of this disease and may suggest in this particular case a cure of the GBM."¹¹ To write "cure" for GBM was quite dramatic.

What did this patient's story teach us? That maybe this treatment could work. What if we had injected her "better"? What if the gene transfer had been more efficient? The optimistic lesson from this case was that it was all about the efficiency of gene transfer. Efficacy called for efficiency (of transfer). How can this efficiency be achieved? Then came this crazy idea: why work with defective vectors, which were dead after their single shot? Why not inject replicative vectors? Any locally transduced tumor cell could then produce more viruses, to infect more

surrounding cells, and so on and so forth. Tempting, but what about safety? Well, these patients have a median survival of 8 months. Would they really care about possible leukemia that could potentially be controlled with chemotherapy and could be deadly in years? And any infected virus-producing cell would express the suicide gene, the potential leukemic cells also, making them targets for GCV. I turned this around and around in my mind, and realized that it would all be about efficacy in experimental models. If it was not that much more efficient in mice, that would be the end of the story. If it was, however, then it would be time to discuss safety with experts. And so we started. And it worked nicely in mice. We submitted an abstract to the ASGT and were rather disappointed not to be able to report our work in an oral presentation. Although many researchers had started long ago to make RCR for various purposes (reviewed by Dalba *et al.*^{12,13}), obviously, RCRs for a clinically oriented development were low in the pecking order.

And so it was that we met with Nori. Being “parked” together likely helped build our collaboration and friendship. We could have been competitors (we had both applied for patents at almost the same time), but we collaborated. We published articles reporting the amazing efficacy of these vectors.^{14,15} We reported improvements in use, such as transduction through DNA gene transfer of replication-competent genome. And we did not overlook safety. Being half worried that the regulatory authorities would not allow the use of RCRs, I developed the concept of semi-replicative vectors.¹⁶ These are two complementary vectors that can produce only half of what is necessary for replication, gag/pol or env, plus a therapeutic transgene. They beautifully (yes, beautifully, this is how I saw it) spread *in vitro* and *in vivo* and “never” recombined to form fully replicative vectors. We also did some preclinical safety studies showing that RCRs could not be detected in the periphery. However, obtaining authorization for clinical evaluation appeared far away.

But then light emerged at the end of the tunnel. Nori met with Doug Jolly, one of the pioneers of suicide genes who well appreciated the concept. Soon, a company was formed (Tocagen) and, yes, successfully raised the big money needed to start the endeavor. That there were people willing to invest on RCRs at the time was quite amazing to me. It could not have happened here in France. Nori was involved, but the deal of these entrepreneurs with my university, which holds my patent on this work, never worked out. The preclinical work had been completed, and it was now time for clinical trials. No way could I do it on my own and

compete with this company, so I gave up, regretting that I had been unable to bring this concept all the way to the clinic.

The vector was optimized and produced under GMP. The Food and Drug Administration authorized a first clinical trial, with no major difficulties, as far as I know! I doubt it would have been that easy here. Forty-five patients with gliomas were treated.¹⁷ The treatment was well tolerated and improved survival, opening the way to a Phase 2/3 trial. But that’s not all. Tocagen investigated whether it would be possible to administer the RCRs intravenously! Their preclinical data in rodents did indeed support this route of administration. The vector reached the tumor and efficiently spread within the tumor.¹⁸ Based on these results, a clinical trial investigating intravenous administration of suicide genes carrying RCRs has been approved and is now enrolling patients! At this early stage of development, it is of course difficult to predict how efficient these first-generation vectors will be. I am quite optimistic that they will show some efficacy and lead to the building of increasingly efficient second- and then third-generation RCRs.

What can be learned from this story? In my first scientific life, when I was working on human immunodeficiency virus, I was lucky to learn science in an incredibly diverse environment, where you had to be multifaceted, doing immunology and molecular biology while also working with sociologists, anthropologists, economists, journalists, politicians, lobbyists... That was quite an education. In my second scientific life, I still do not know whether I should consider myself lucky to have entered the field early. You also had to be multifaceted, but this time through learning about production, regulatory affairs, business, and more, all of which are important but somehow less fun, for me at least. At the time, translational gene therapy called for resilience, adaptability, hard work, and much more for return on investments (academic I mean) that were often low! Having now turned to protein-based biotherapy,¹⁹ I appreciate even more the handicap from which gene therapists suffer. I remember one of my post-docs from years ago. She was a bacteriologist. The bacteria were grown overnight, and it was almost always possible just to put them in the freezer to stop the process and have time off if there was an invitation for a party. Nothing we could afford. Anyhow, the technological aspects of gene therapy, the smartness that can be put into it, are still thrillingly interesting to me. So, I have not fully betrayed the field, because my now preferred protein, interleukin-2,²⁰ can be administered (advantageously?) by viral vectors! And we are exploring

this avenue of research.^{21,22} Another lesson is that much (everything?) is about devoting enough time to good, deep, independent thinking. Think, think, think, think your own thoughts. And finally, it is also very much about people—those you work with and those you work for, the patients. My only wish now is that these RCRs, this generation, and the many improved generations to come will save many lives.

To conclude, let's not forget what Protagoras taught us: that there are always ways to “make the

worst case the better” or, to put it another way, to transform a weakness into a strength.

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