“But Dr. Old, we already have an antibody!”
Reflections on Lloyd Old’s “academic biotech” approach for targeted antibodies

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When Dr. Lloyd J. Old referred to the goals and objectives of his laboratory of Human Cancer Immunology, he typically just said, ”A major objective of the laboratory is the identification of suitable targets for cancer immunotherapy with monoclonal antibodies and vaccines.” This key objective remained unchanged for more than three decades, and I think I have referred to this sentence uncountable times in our scientific reports, in grant applications, in manuscripts, presentations, or in just talking about the laboratory. What at a first glance apparently seems a ”simple” and “narrow” objective actually perfectly describes his quest for understanding the immune system and utilizing its enormous power to control cancer. This sentence is rooted in the four key questions of cancer immunology that Lloyd Old outlined when he began to consider entering this field in 1958 and that he continued asking throughout his entire life:
1. Does the immune system recognize cancer as foreign?
2. What are the targets of recognition?
3. Does immune recognition lead to tumor protection?
4. How can immune protection be strengthened?

He had a lifelong fascination with the extraordinary power of the immune system to make the finest distinctions between molecules (antigens) and thus potentially between normal cells and cancer cells. In the 1970s and 1980s, this power of distinction was most inherent in antibodies, which could do so quickly and with great sensitivity. Based on this idea, his laboratory developed during this time potent serological tools to identify molecules on cancer cells that could be recognized by antibodies and began the challenging process of characterizing and dissecting the antigenic systems on the cellular, tissue, and molecular level. The principle of his approach was “let the immune system decide what antigen it would recognize on a tumor cell and then carefully analyze the immune response and find those antibodies that show selective reactivity with tumors.” Practically, this meant that mice were immunized with tumor cells, monoclonal antibodies (mAbs) were generated from the immunized mice, and the mAbs were then subjected to elaborate screening for reactivity with the cell surface of a broad range of living tumor cells.

Using this approach, a huge panel of antibodies and antigenic targets was identified for a large variety of solid tumors, the tumor stroma, and tumor endothelium. The antibodies with the highest selectivity for cancer were then prepared and studied in early-phase clinical trials in cancer patients. For an antigenic system to become really exciting, a high degree of specificity was of utmost importance to Lloyd Old, and his quest for specificity became legendary. More then a dozen of these antibodies have been licensed by large and small pharmaceutical companies and are in various stages of clinical development for the treatment of patients with cancer. This is unrivaled for a single academic laboratory, and when this success was to be pointed out by someone, quite atypical for him, he could not resist occasionally showing some pride in his unique and industrious “academic biotech” approach.

How did I get involved in Lloyd Old’s antibody program? For me, it all began with a phone call. One evening in early fall of 1993, my phone rang at my then home in Italy. The caller did not identify himself initially but just said “Hello” in a very unmistakable intonation. I had not really talked with Lloyd Old since I left his and Herbert Oettgen’s laboratory at Memorial Sloan-Kettering Cancer Center (MSKCC) in New York about three years earlier to pursue an exciting scientific endeavor in Italy. But in an instant I knew who was on the other side, playing one of his little phone games by trying to coax the person he called to guess who would be on the line. At that time, he called to invite me to come back to New York and join his just three year old unit (later the New York Branch) of the Ludwig Institute for Cancer Research (LICR) at MSKCC to pursue research in antigen-specific cancer immunotherapy. And involved I got, hands-on from day one in the purification of a humanized antibody (huA33) for the first clinical trials in patients with colon cancer. But more about this antibody later.

Lloyd Old loved a good one-liner, idiom, proverb, or pun. He drew on them frequently and sporadically repeated them in a mantra-like fashion to underscore or illustrate a key point, bring out the quintessence of a topic, or just help an audience better remember or connect a scientific or historical fact or view. He made use of them to hold up a mirror or courteously tease himself or the team, but also occasionally to convey what seemed absurd or bizarre to him as it presented an unnecessary hurdle to a development path that was so straightforward to him but took years for others to comprehend. There are still many of these one-liners and the topic they are associated with that come into my mind, several of them uttered many years ago—but these are the memories a fine teacher and mentor leaves you with. Hence, I selected a few that I personally associate with him and his vision and stewardship of the targeted antibody cancer
immunotherapy program of the LICR. Though these are just some vignettes, they are representative of some of the cornerstones of the program he built. If they are not cited as you may recall them, just keep in mind that, in his hands they were highly adaptable tools, always applicable to whatever he wanted to illustrate, and he made them fit a particular occasion today and another tomorrow.

“But Dr. Old, we already have an antibody!”

(“His view on “failure” of mouse mAbs; need for establishing the principles of antibody therapy using many different antigenic systems; first CRI symposium dedicated to mAbs to overcome current challenges of the field)

This remark reminds me of the differences in Lloyd’s view of the field of cancer immunotherapy with mAbs and the rest of the world’s, especially traditional pharmaceutical companies that started to embrace mAbs in the 1980s and early 1990s. It represents a typical example of his capability to look ahead a decade or more to how a field may evolve, to see what is truly important, and to recognize potential and connections others would not even think of contemplating at that time. He also liked to use this phrase now and then with a sense of irony, conveying his disappointment or irritation, especially when non-scientific reasons prevailed and hindered the—for him so obvious—path of development of a therapeutic antibody. For me, this remark also stands as a symbol for the general scientific and commercial mindset during the first phase/generation of therapeutic antibodies.

The early 1990s was a time of major changes in the field of therapeutic antibodies. The hype of the first mouse mAbs (“magic bullets”) had come to an end as the rush into clinical studies proved to be quite sobering. Although for a few antibodies some basic principles could be established, such as actually reaching the tumor site or causing inflammation at the tumor site in a cancer patient, mouse mAbs turned out to be “really” highly immunogenic in humans, allowing potentially one round of injection of a mAb before the patient’s immune system mounted a strong immune response neutralizing the injected murine antibody. This immunogenicity was often associated with severe side effects, precluding not only further administration of any therapeutic antibody but also the ability to thoroughly study an antibody in the environment that really mattered—the cancer patient. Furthermore, in some cases the specificity of mAbs and their antigenic target distribution had been very poorly defined prior to their first clinical explorations, resulting in disastrous adverse events in patients treated with these antibodies. As a result, the field of antibody-based therapies for cancer was widely believed not to be living up to the expectations. Lloyd Old, who pioneered the clinical use of mAbs for cancer therapy, rebutted this view. To him, this assessment was clearly premature and was not based on any demonstrated or inherent limitation of the true potential of this immunotherapeutic approach, but rather on the unrealistic expectations and time scales that so many people placed on mAbs. As with every novel approach, there had been technical and logistical challenges, and these difficulties just outpaced clinical successes. What was needed, in his opinion, were clinical studies that were well designed and asked fundamental questions on biopsy-based antibody biodistribution and kinetics with well-characterized and specific antibodies against a much broader range of antigenic targets—not only antibodies against antigenic targets on the surface of cancer cells, but also to antigens found in the immediate tumor environment such as the tumor stroma and tumor endothelium—to properly identify and establish the principles of antibody-based tumor therapy. Discussing his view at that time and stressing the need for more and new antibodies and targets with a pharmaceutical industry partner, Lloyd allegedly received the reply, “But Dr. Old, we already have an antibody!”

To overcome these challenges and to bring the field into the next decade, he inaugurated in 1993 a cancer immunology symposium series organized by the Cancer Research Institute (CRI), in which initially during alternating years, the focus was on antibody- or vaccine-based immunotherapy. He strongly believed in the power of the academic mind, and he brought together academic scientists and clinicians that shared with him the view that the field needed to be driven by asking clear basic scientific questions about an antibody and its antigenic target first before embarking on therapeutic trials with this reagent. In the first CRI antibody meeting in 1995, Lloyd Old strongly voiced his critical view outlined above in his opening address, and it left its mark. The CRI symposium series is now soon entering into its third decade and has evolved into one of the most highly regarded and influential scientific meetings in the field of cancer immunology. As was typical for him, rather than giving up on something he was deeply convinced in, he regrouped and reorganized his strategy and forces until there was progress and the next goal could be defined.

“Every antibody has its warts”

(Quest for specificity; critical importance of immunohistochemistry and pathology; patient safety first)

Lloyd Old’s unwavering principle for any meaningful cancer immunological intervention—antibody or vaccine—was specificity. In regard to therapeutic antibodies, specificity referred to both the selective expression of an antigen and the discriminating capability of an antibody to recognize a defined antigen in vitro and in vivo. Before any of the antibodies could become a candidate for first clinical exploration, an antibody was subjected to the most thorough specificity analysis, including a comprehensive in vitro assessment of its serological reactivity profile for a broad range of human normal tissues, cancer tissues, and numerous cultured cancer cell lines. Immunohistochemistry (IHC) was his technology of choice and it left its mark. The CRI symposium series organized by the Cancer Research Institute (CRI), in which initially during alternating years, the focus was on antibody- or vaccine-based immunotherapy. He strongly believed in the power of the academic mind, and he brought together academic scientists and clinicians. As was typical for him, rather than giving up on something he was deeply convinced in, he regrouped and reorganized his strategy and forces until there was progress and the next goal could be defined.

Lloyd Old eagerly waited for the IHC results of a given antibody, paying greatest attention to any flaws he could detect for an antibody. No meeting was complete without at least some projected histology slides that could be admired and discussed. Although passionately searching for the perfect antibody, he knew that this was more a dream than reality, because to him, “every antibody has its warts”—one just needed to look close enough. However, a wart was for him more like a cosmetic problem that could be dealt with and not something vital that resulted in the abandoning of a particular antibody, because this wart would not be bearing major potential risks of side effects for patients to be treated with this antibody. A good example for an antibody’s warts would be the binding of the kidney cancer antibody cG250 to the normal bile ducts. If a small dose of
unlabeled antibody cG250 is given prior to the injection of a therapeutic or diagnostic dose of radiolabeled cG250, the sites in the bile ducts are occupied and blocked with inert unlabeled antibody, and the more abundant antigen sites in kidney cancer cells can be targeted with high precision with the active, labeled antibody.

“In vivo veritas”
(Unexpected immunogenicity of humanized Abs; chimeric vs. CDR-grafted mAbs; prediction of their potential immunogenicity)

The LICR targeted antibody program under the leadership of Lloyd Old had developed its own unique model to efficiently study and evaluate the potential of an antibody for further clinical development. The objective for the first-in-human studies was to gain as much data from a single trial about an antibody’s safety, immunogenicity, targeting, pharmacokinetics, and, if possible, anti-tumor activity. The second generation of antibodies had all been humanized to make them potentially less immunogenic and thus hopefully avoid the same fate as the first generation of murine antibodies. This new antibody humanization know-how was not available within the Institute. Through collaborations or partnerships with specialized antibody engineering biotechnology companies, the various mAbs in the Institute’s portfolio were chimerized or humanized using the partner’s “state-of-the-art” technology.

However, the immune system could not always be fooled as we unfortunately learned quite rapidly from the first clinical studies with the humanized antibody A33. In some patients, the trace-labeled antibody cleared very fast after repeated rounds of the injection. We subsequently developed highly sensitive and specific assays to measure human anti-human antibodies (HAHA) that allowed us to monitor patients in real time and to determine when a patient would need to be taken off study as further treatment may have a high likelihood of a severe adverse event. Applying these HAHA measurements to all our clinical studies, we were quite surprised to learn that, of the five different antibodies we had in clinical trials at that time, the two CDR-grafted humanized antibodies were more frequently immunogenic in patients than the three chimeric antibodies, which were generally believed to be more immunogenic because of their low-tech conversion and their higher degree of mousiness. So, whenever a new antibody humanization or antibody de-immunization technology was developed or proposed and enthusiastically being presented to Lloyd Old, he would carefully caution us to wait and see what happens when the antibody is repeatedly being injected into patients—in essence, and in his own words, “in vivo veritas.”

“A bird in the hand is worth two in the bush”
(LICR antibody GMP production facility; clinical trial with minimal amount of reagent)

A cornerstone of the LICR antibody program was the capability of manufacturing its own clinical grade cGMP-compliant mAbs. For Lloyd Old, this capability was of utmost importance. It allowed the Institute to break the circle of dependence on pharmaceutical companies, who then had the monopoly on generating clinical grade reagents, especially expensive biologics such as mAbs. As a result, researchers who developed an antibody in the laboratory typically had to hand over a promising reagent to a company, which subsequently developed a clinical grade reagent and conducted the clinical trials, usually with little input of the discovering scientist. Alternatively, clinicians interested in early-phase clinical trials with antibodies were approached by a biotech or pharmaceutical company and could participate in a clinical trial designed and sponsored by the antibody controlling company, but again usually with little intellectual input from the clinical investigator’s side. This situation was not going well with Lloyd Old’s ideas of a clinical-targeted antibody cancer program in the Institute. For him, the first-in-human clinical trials were the last phase of the discovery process and not the first phase of new drug development. They should be conducted closely with the discovery team, which included clinicians. To accomplish this, obviously having control and ample supply of the study agent was needed. His motto was, “He who owns the reagent is he who owns and controls the clinical trial.”

Accordingly, he implemented in the Institute various building blocks, including: an active program to patent the antibodies and the antigenic targets to give the Institute more protection and power of leverage of its intellectual properties; a GMP production facility for the manufacture of clinical grade mAbs at the LICR Melbourne Branch; a tight international network of scientists and clinicians, including radiochemists and clinicians from nuclear medicine; and an office of clinical trials management to help coordinate and manage all aspects of the clinical antibody program. The latter included managing the regulatory approval process for the antibodies to be used in clinical trials and ensuring that the clinical trials are in compliance with all applicable standards, regardless of where the clinical trial would be conducted in the world. He was particularly proud of the Institute’s unique and successful GMP manufacturing capability. The capacity of this academic facility was naturally limited to smaller production lots, and with the expanding clinical antibody program, the facility was under pressure of keeping up with the timelines and demands especially for antibodies with low production yields or when a longer production process development was necessary. Alternative ways of antibody manufacturing were discussed, and some antibodies were outsourced for manufacturing, which generated a whole new set of challenges and uncertainties.

Recognizing this and also the fact that the financial resources for the program were limited, Lloyd Old liked to remark, “A bird in the hand is worth two in the bush.” For him, it was clearly preferable to have a small amount of reagent and to be able to address the basic question only, than to have to wait a long time for potentially larger amounts, or risk not being able to perform a clinical trial at all.

“They will disappear like snow in the hot desert sand”
(Healthy skepticism toward changing what nature has perfected by genetic engineering just because it is possible)

Though fascinated by the huge potential of antibody molecular engineering, Lloyd Old at the same time expressed a healthy skepticism toward changing what nature already had designed and shaped throughout eternity. Not everything that could be engineered and modified in an antibody was indeed as meaningful to him as claimed. Single-chain variable fragment (scFv) antibodies, a small construct maintaining the antibody binding properties of a full-length antibody, were no exception. In the early- and mid-1990s, there was some widespread doubt that a full-length antibody, because of its large molecular size, could target and penetrate sufficiently into a solid tumor mass to potentially have therapeutic efficacy. It was proposed that smaller molecules such as the newly engineerable scFv antibody constructs would easily overcome this perceived problem. In his opening remarks at the CRI antibody symposium in 1998, he addressed this issue by commenting, “Once injected, scFv antibodies will disappear like snow in the hot desert sand.”
rightly explained that these low molecular weight constructs would be cleared rapidly through the kidney and only a small amount of the injected dose would ever reach the tumor.

“Just imagine how it could burn a hole in the carpet”  
(Allergy at the tumor site)  
Recognizing the natural limitations of a naked antibody for targeted immunotherapy of cancer, Lloyd Old was always thinking of how to improve the potency of antibodies. In his typical manner, his ideas were going beyond the usual suspects such as the more common payloads including radionucleotides, toxins, or cytokines. He had suffered from severe allergies throughout his life, and obviously he had experienced first-hand the power of the immune system. One day we were walking down from his office to the conference room with a colleague, who had been designing and generating the next generation of antibody constructs for the collaborative targeted antibody program, when Lloyd Old threw out the idea of an IgE construct for one of our antibodies. Musing about this approach and the idea of selectively triggering the most powerful inflammation reactions a natural antibody is capable of at the tumor site, he smiled and commented, “Just imagine, how an IgE antibody could burn a hole in the carpet!” He was a cornucopia of ideas that he freely shared with everyone, regardless if a person was from academia or from industry, if a person was close to him or if he met that person for the first time. Unleashing through an antibody a controlled allergic reaction at the tumor site is just one of his many ideas still up for grabs.

“We can talk about this ‘til the cows come home”  
Lloyd Old loved to talk about science, to share and discuss his vision in greatest detail, and it was well known that meetings (and not only those about antibodies) that he chaired typically went long over the scheduled time. However, he also could express his impatience about too much talk. Especially if, in meeting after meeting, an idea, a project, or a program was belabored without being implemented into practice or without showing first experimental results or concrete steps forward at the next meeting, he would utter, “We can talk about this ‘til the cows come home.” To him, an idea or hypothesis needed to be put into practice and validated by sound scientific experiments, and only actual results, reproducible and independently confirmed, could validate an idea. Concluding in his own words, “The proof is in the eating of the pudding.” In this sense, his antibody program was not only to him the tastiest of all pudding.

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