

Novel Findings To Establish A New Generation Of Breast Cancer Diagnostic And Therapeutic Immunoconjugates

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My longterm project aims at gathering essential information about tumor infiltrating B cells (TIL-B) that accumulate in cancerous tissues. Using results from my previous research, I investigated the potential of the B cells in breast carcinomas to be a source of recombinant antibodies for tumor diagnostics and therapeutics. The definition for the related tumor associated target antigen has huge clinical importance. My previously selected tumor binder antibody fragment was successfully processed in terms of the necessary transformation and as a part of the purification and conjugation steps. Due to its unique ganglioside specificity with central functions in tumor progression, the resulting immunoconjugate is of great importance. The study of TIL-B cell immunoglobulin variable gene usage and the antibody fragment phage display libraries answer important tumor-immunological questions. The results led to a new generation of tumor-specific immunoconjugates for early cancer diagnosis and effective targeted therapy.

1. Scientific Background

1.1. “If One Door Closes, A Window Will Open“

Against assorted cultural, financial, and economic backgrounds, there are great differences between jobs and work possibilities. Being an enthusiastic biologist in a rather poor country in Central Europe, Hungary, I was never afraid of difficulties with work-infrastructure and the hard, steadfast “fight” for grant supports. But being a female scientist, I always had to face the problem of trying to harmonize overwhelming obligations both at the institute and at home. Although I have been trying my best for years, it seemed I failed in this struggle... But on the very day of going through a divorce I never wanted, I received the envelope with my Fulbright Grant acceptance! *So for me it seemed to be very true: „If one door closes, a window will open!“*. This new scientific challenge and possibility gave me great strength to keep going ahead and to focus on my scientific project with a very clear mind (even if with a broken heart) (Fig. 1).

To tell the truth, I felt really honored that my tumor-immunological project, the

basis of which I had been building up brick by brick through extreme infrastructural and financial difficulties, was found scientifically valuable and would now get a new boost through the Fulbright Grant and a novel host institute. In order to answer the project-related questions summarized in the “Objectives” and to go ahead with the project, *a great arsenal of molecular, genetic, and biotechnological techniques and instruments were needed*. The Department of Glycoimmunotherapy at the John Wayne Cancer Institute has been well known for its scientific achievements in glycochemistry and for its immunotherapeutic interests. It seemed that a great mutual scientific interest coupled with an effective scientific work period would result in successful achievements, leading to further cancer diagnostic and therapeutic potential. As I have already known the host Institution’s Department Head, Dr. Mepur H. Ravindranath, through previous scientific communication, as well as lectures and publications, I was really happy about this possibility.

The project was based on my novel idea and the original “proof of principle” that I could demonstrate in a special type of breast tumor. In the course of my Fulbright project, important preparatory work phases for tumor-specific antibody development and new immunoglobulin studies in other breast tumors were set down as tasks.

This research field deals with a highly exciting question in tumor immunology. Not only because it can reveal novel cell surface structures that specifically characterize the cancerous cells, but also because it

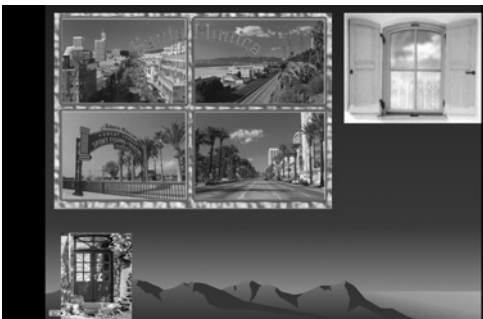


Fig. 1. The Fulbright Grant opened a new window, a new possibility for my work.

provides cancer diagnostics and targeted therapeutic drugs.

It is important to know that generally all solid tumors could be characterized with any immune cell infiltrates. Researchers investigated only those cells that were available in large amounts. The novel methodological approach that I developed reveals and investigates those special cells that are available only in tiny amounts (Fig. 2.).

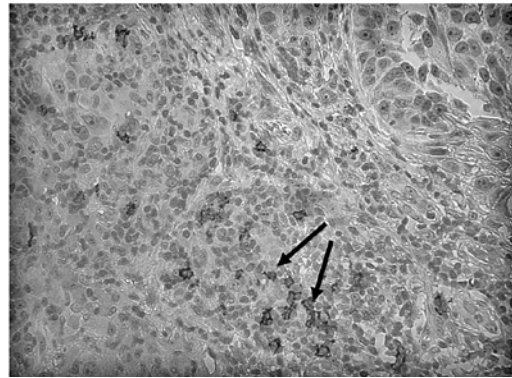


Fig 2. B lymphocytes stained by immunohistochemistry in cancerous tissue

My original hypothesis was that the tumor tissue itself contains the genetic information, which has to be revealed and then processed further. Special immune cells infiltrating the cancerous tissue have to be investigated through a detailed DNA analysis and tested experimentally against the original tumor. All this serves as a new approach for novel tumor diagnostics and therapeutics (Fig. 3).

The importance of the project is the development of tumor reactive molecules, being potential candidates for tumor diagnostic and therapeutic usage. The immune system is responsible for producing the defense molecules against the invader that attacks the body. These molecules are called antibodies, immunoglobulins produced by B cells. My project aims to find and further develop the “anti-cancer-bodyguards”, immunoglobulins that specifically recognize the inside attackers, the cancer cells, and which have the potential to eliminate them. Techniques in molecular genetics and biotechnology made it feasible

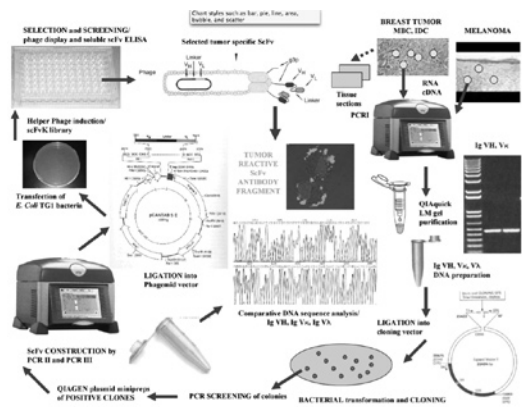


Fig 3. The flowchart diagram of the main experimental workphases

Fig. 4. The „wandering circus” as I called my working background.
Whole project work background : The „wandering circus”

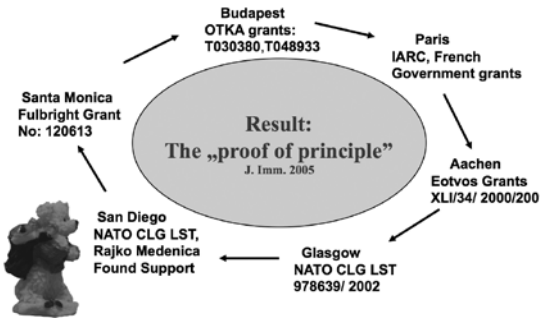


Fig 4. The „wandering circus” as I called my working background



Fig 5.
I arrived at the JWCI with all my scientific plans and preparations

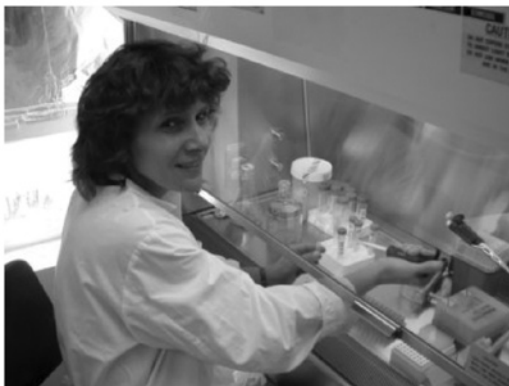


Fig 6.
I was working hard to be able to start with the real experimental works

to produce and investigate different fragments of the antibodies. *Antibody fragments are ideal tumor-targeting reagents*, as they retain binding capacity, and they are intermediate-sized multivalent molecules which provide rapid tissue penetration, high target retention, and rapid blood clearance. A further advantage is that antibody fragments can be conjugated with labeling or cytotoxic drugs, essential for diagnostic or therapeutic use, respectively.

The whole project’s working background is my „wandering circus”, as I called it in one of my lectures, referring to how I did the research work to obtain the results (Fig. 4). For years I have been writing several applications to obtain Hungarian and international scholarships and grants. As the project was interesting for institutes abroad, I could always find the necessary infrastructures and instruments I needed for the different work-phases. In the course of the Fulbright Grant, another new Host Department was opened to me. Since I was planning a huge project, I had to prepare myself well. So, by the summer of 2006 I already had some important starter preparations made, and I had selected several necessary DNA samples and clones.

1. 2. Objectives

My main objectives were to explore further the molecular genetic and immunological characteristics of the special immune cell type, accumulated

in tiny amounts in breast carcinomas. I wanted to acquire a broader knowledge concerning the antibodies produced in different breast carcinomas in order to provide further support to my original “proof of principle”. Lastly, I hoped to reveal the potential for tumor diagnostics and therapeutics of the immunoglobulin variable genes in question, with defined tumor-associated antigen specificity, since this is of high importance.

2. Working Days Under My Fulbright Period

2.1. Make the most of the Fulbright Grant

I arrived at my host institution, the John Wayne Cancer Institute, with all my scientific plans, a great many documents, and starter biological preparations (Fig. 5). However, soon after a hearty welcome by the Glycoimmunotherapy Department Head, my supervisor, Dr. Mepur H. Ravindranath, I had to face a problem. After a thorough check in the roomy laboratory, I saw that no instruments were available for the molecular genetic and biotechnological work I planned, but only for the cellular and biochemical studies. Of course, I was hoping to get to the biochemical phase of my studies, but these methods were scheduled for the very last period, after I had accomplished all the first ones. So, in the next days and weeks I went searching for the necessary instruments, consumables, and reagents that I needed. The various processes necessary for my Fulbright

project required special infrastructure of a technical nature. Because of the numerous methodological phases, it took quite a lot of time to gather all the necessary information through the Internet and through catalogues, which I used to order the equipment that I needed. I inquired in nearby laboratories about which instruments might be borrowed or used somewhere else. The list of the necessary items to order was too long! Even though my supervisor did his best and went to great efforts to make arrangements for me. However, with a lot of additional work, patience, and the support of my supervisor, I succeeded in building a laboratory environment for molecular genetic and biotechnological research. In the first part of my stay a great amount of time was needed to make all the necessary arrangements and changes in the laboratories: obtaining the equipment, setting up and calibrating the instruments, sterilizing the consumables, and ordering the chemicals. *As a result of my “input”, I have now set up well functioning laboratories for all kinds of molecular work, and one for bacterial and phage display (Fig. 6).*

During my Fulbright period I was really very busy, and I learned a great many different things, important both for work and for life. The institute required some courses and exams for handling biological materials, hazardous chemicals, and radioactive materials. I attended the courses and passed the online exam for the National Institute of Health (NIH), concerning goals and principles of human participant protection, as all cancer related experimental work requires these certificates.

As I wrote applications and registered for US conferences related to my scientific interests, *I managed to take part in several meetings, important in cancer and antibody research.* I presented results from my previous work as well as new data at five conferences, and I attended a workshop organized by my host institute. My scientific work could be discussed and collaboration could be built at these conferences. I heard lectures that were highly interesting and important, which summarized the current state of scientific knowledge in cancer biology and therapy, which is vast, and I also learned about antibody engineering technology. So, my Fulbright stay gave me the opportunity to take part in the AACR Frontiers of Cancer Prevention Research conference in Boston, November 12th–15th, 2006, where I displayed a poster presentation, entitled: “A novel approach to generate tumor-specific human immunoconjugates from breast carcinomas and melanomas”. This poster was accepted for presentation at the highly important Antibody Engineering Meeting, held in San Diego (December

5th – 8th, 2006). This provided me with a good opportunity to hear about the latest news and technical improvements in this field (**Fig. 7**). The “Chemistry in Cancer Research” international meeting in San Diego (February 4th – 7th, 2007) gave a detailed picture of the cancer therapy drugs and their effects.

One of the greatest experiences for me, though, was to take part in the Centenary AACR (American Association for Cancer Research) Meeting in Los Angeles (April 14th – 18th, 2007) (**Fig. 8**). That was a once in a lifetime event! A hundred years of efforts were celebrated and about 17,000 scientists gathered to share their ideas and to develop novel ways to reach the final goal: curing cancer. I feel a little proud that it was at this very international conference that my abstract was awarded the 2007 AACR-Avon Foundation Scholar-in-Training Grant. The title of the poster I presented was: “Novel findings obtained by single chain Fv antibody library establishment from tumor infiltrating B lymphocytes of human breast carcinomas and melanomas” and the abstract was

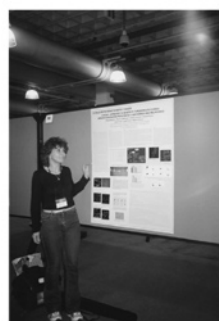


Fig. 7. Travelling to international conferences and presenting the work



Fig. 8. Just before the great AACR meeting organised in LA Convention Center

published in the Conference Proceedings Book (**Fig. 9**, **Fig. 10**, **Fig. 11**). It was a good feeling that so many researchers were really interested in my presentation, and I could also meet with and show my new data to my previous collaborators from all over the world. I was glad to meet there the Hungarian melanoma tumor progression expert, Professor Dr. Jozsef Timar, in whose department I would continue my research in Hungary. I also attended all the ceremonial events, the AACR Centenary Concert in the Hollywood University Studios Concert Hall, a benefit to support cancer research. Anyhow, of highest value for me was the fantastic scientific lecture program, where I could get detailed information about the landmarks in cancer research in the last 100 years and the latest achievements. Some further important new collaboration possibilities were built for my research there also.

I was asked to give two lectures at JWCI about my research work and about the poster I presented at the AACR in April. I gave a lecture at UCLA about the Hungarian cultural, historical, and political

characteristics of my work. It was of great interest among the students, and that was shown by their questions and the feedback I received later. I was given the chance to attend a special workshop organized by JWCI entitled “Antibodies in Melanoma”. It was of special interest for me as its main topic was those tumor-associated molecules I am highly interested in.

2.2. *Experimental work*

As a joint possibility that arose at the San Diego meetings, through an opportunity given by previous collaborators, *I could go ahead with very specific experimental work*, until the necessary conditions could be arranged in the Department at my host institution. During the day I attended the scientific lectures at the meetings, and then I would work with my experiments until very late in the evening. It was only through this possibility, arranged with the collaborating foundation, that I could go ahead with most of my planned molecular work. Accordingly, immunoglobulin heavy and light chain variable region genes were successfully amplified from the rare medullary breast carcinoma and the

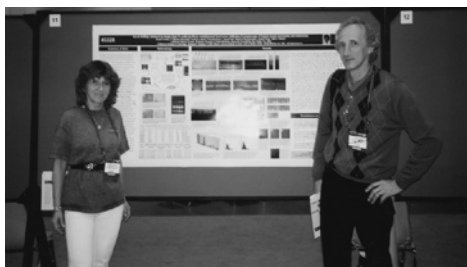


Fig. 9. My poster was presented and awarded



Fig. 10. The AACR-Avon Foundation Scholar in Training Grant celebration event

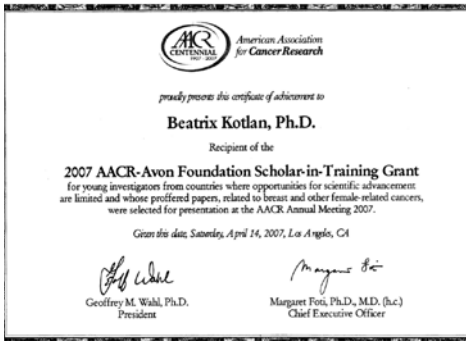


Fig.11. The AACR Avon granted abstract was published in the Centennial Meeting Proceedings Book

general invasive ductal breast carcinoma tumors. I managed to make some further purified DNA preparations, which were now ready for further bacterial work (ligation into suitable cloning vectors, and E.coli TG1 bacteria transformation and cloning), DNA sequence analysis, and antibody fragment construction (**Fig. 12. A**).

After the cloning process *insert positive clones were defined by PCR screen and purified thereafter with a plasmid purification kit (Fig. 12.B)*. Samples were controlled by quantitative and qualitative analysis. As a result, I had hundreds of immunoglobulin heavy and light chain inserted clones ready for the DNA sequencing reaction and comparative DNA analysis. I went on with the DNA sequence reaction and analysis work from some selected clones, using specific programs: Chromas, BioEdit, Clustal W, and TreeView. It was hard work, far too time consuming and work-intensive, especially because I did not have some important software

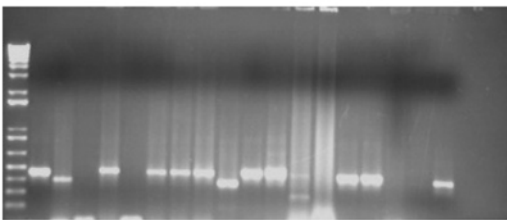


Fig. 12 A:
IDC breast tumor originated Ig VH insert positive clones obtained by PCR screening (qualitative gelelectrophoresis)

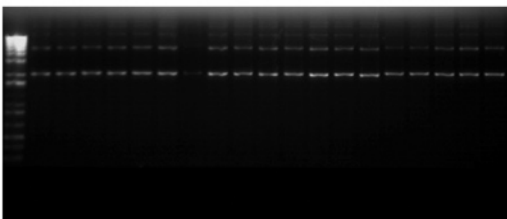


Fig. 12 B:
Qiagen plasmid minipreps / cloned breast carcinoma originated Ig VH inserted vector (qualitative gelelectrophoresis)

to do my analyses, as this software was not available for my studies. In defining specific primer usage, chain orientation, homology levels, subgroupings, and mutation rates, etc. are only a small part of the tasks that have to be accomplished for a full immunoglobulin repertoire analysis. The comparative DNA sequence analysis with different accessible databases (IMGT, Blastn) was also strenuous (**Fig. 13, Fig. 14**).

Later on, thanks to the above detailed possibilities, I could also go ahead with another “working package”, that is, with my previously selected anti ganglioside antibody fragment. What this means is that this special scFv was ligated into the pCANTAB/ E.coli TG1, HB2151/ with the RPAS system (Amersham Bioscience) for specificity testing and into the pET 26b vector/ BL21, NovaBlue, BL21 (DE3), BL21 (DE3) pLysS bacterial system (Novagen) for purification (**Fig. 15**).

The soluble scFv antiGD3 antibody fragment in pCANTAB vector was tested with a chamber slide immunofluorescence technique against breast tumor and melanoma cells. I cultivated and processed for further binding assays several breast tumor (*MDA MB-231, ZR751, MCF7, IDC TU No1, IDC TU No2*) and melanoma (*SK-Mel 28, Mel 24, HT199, HT168-M1*) cells lines. Several tumor cell membranes were also prepared, which preserved the antigenicity and were ready for enzyme labeled immunoassays (ELISA) for antibody phage library selection. This is important, as it is essential to be able to test the previously selected, purified antibody fragment preparations and to screen any further antibody fragment libraries. As a “point ahead” result it is of great importance to see the retained specific binding capacity of the purified anti ganglioside antibody fragment on breast cancer and melanoma tissue. The immunofluorescence assay

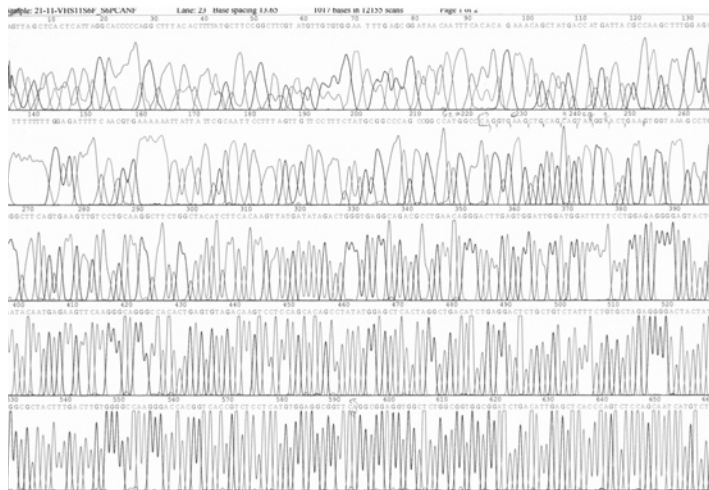


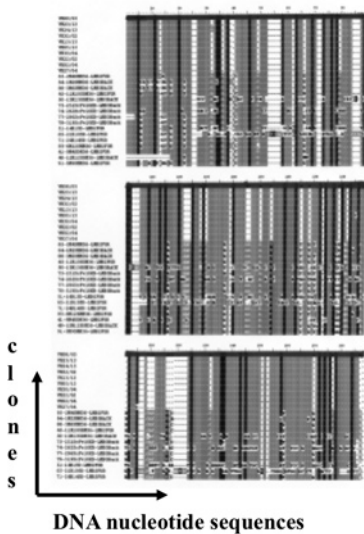
Fig. 13. Representative DNA sequence reaction result of new Ig VH region

and the chamber slide technique gave excellent samples for the confocal laser microscopy analysis (fig. 16A.). The final achievements in this topic were the immunofluorescence binding results with the new anti-ganglioside antibody fragment products after the first purification processes against invasive ductal breast carcinoma cells in FACS analysis (Fig.16B.) and against melanoma tissue sections analyzed by laser confocal microscopy (Fig. 17A,B).

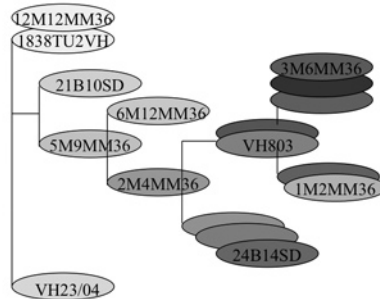
Because of the initial technical problems, the project proceeded more slowly than planned. But with my reorganized work schedule and with the other possibilities worked out for the experiments, the project came into an important phase. By

then the laboratory for biotechnological work-phases was fully equipped and prepared. I did a lot of work sterilizing the necessary equipment and the consumables. In February we applied for prolongation at the CIES, and I received an extension until the 13th of September, 2007, without financial support. The JWCI offered a possibility for my prolonged stay, and they wanted me to join a melanoma project, also. As they were highly interested in my technologies and know-how, I was asked to explain these methodological processes and the necessary steps. I supplied the requested protocols necessary for scFv library generation from spleen samples of patients with melanoma, and I was pleased to be involved in the first experiments.

A/ Homology search by Ig VH DNA alignment



B/ Clusterisation by TreeView analysis and Fitch-Margoliash statistical estimate.



C/ Iimgt/V-quest „Collier de Perles” aminoacid chain similarity

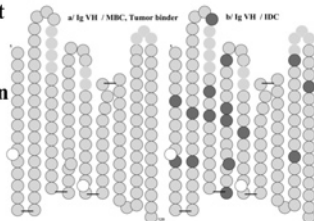


Fig. 14. Comparative DNA analysis

ScFvK antibody fragment inserted positive clones obtained by PCR screening / analytical gelelectrophoresis

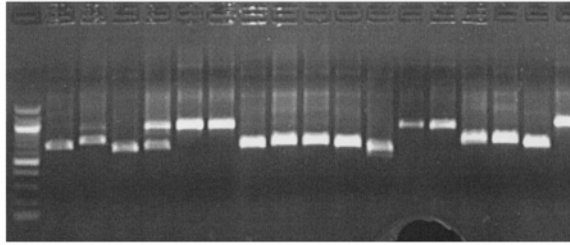
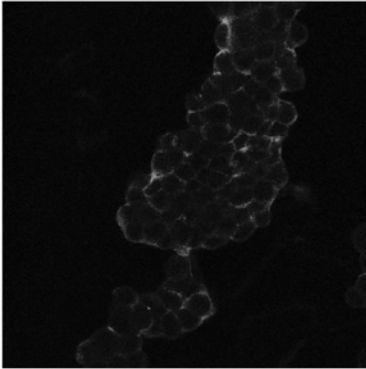


Fig. 15. Successful further bacterial cloning of the antibody fragment of interest

A/ Immunofluorescence with the antibody fragment / MCF7 breast carcinoma / chamber slide – confocal laser microscopy



B/ Indirect immunofluorescence assay / antibody fragment preparations / IDC breast carcinoma (ZR751) cell lines - FACS analysis

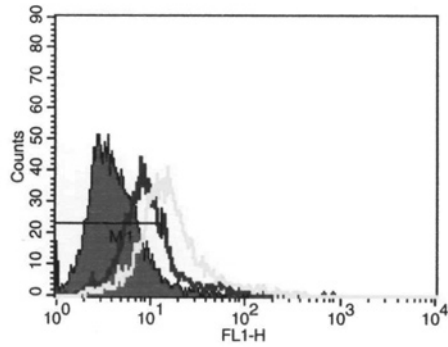
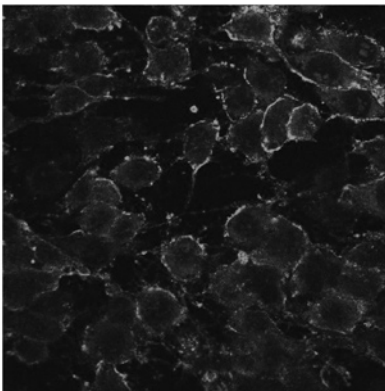


Fig. 16. The prepared antibody fragment reacts with breast carcinoma cells

A/ Immunofluorescence - Confocal laser microscopy / SK-MEL28 melanoma cell lines



B/ Immunofluorescence - confocal laser microscopy / melanoma cryostat tissue section.

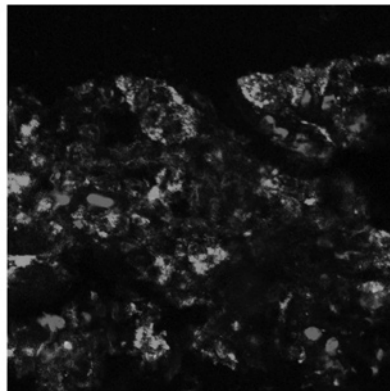


Fig. 17. The prepared antibody fragment reacts with melanoma cells

I was happy with all my investigations. *I was working in the lab day by day, and very late into the nights. Also on weekends and holidays. And my bacteria were working also! The work came finally to its most interesting phase, when scFv libraries could be constructed and tested for their tumor binding capacity. Similarly, the DNA sequencing of immunoglobulin variable region genes would provide a great deal of extremely new information with a strong impact on tumor immunology and novel diagnostic and therapeutic approaches.*

3. Cultural And Sports Events During My Fulbright Period

I will always remember the very first invitation I received for a *get-together Fulbright dinner* at the University of California Los Angeles (UCLA), on October 18th, 2006. I was accompanied by my supervisor, who had been a Fulbright scholar himself, many years ago. I enjoyed the atmosphere, the delicious dinner, and the nice people from all over the world.

There were also some organized parties, although I could not take part in most of them, because of my busy schedule doing experiments. But the few I attended were fantastic and remarkable, especially the ones organized by our wonderful Cultural Referee, Mrs. Ann Kerr (**Fig. 18.**). These events gave always *the nice feeling that we were like a family*. Despite the fact that we all have different backgrounds, *we have a common task: to accomplish our project and give the most of our capacities, as delegates of our home country*. I liked very much the community work to help the poor in Los Angeles with food at Westside Food Bank. I proudly wear the nice sweatshirt we received, remembering the Organizations' Annual Hunger Walk celebrating 25 years of service to the community. The *excursion to the RAND Corporation*, a nonprofit institution that helps to improve policy and decision-making through research and analysis, was very interesting. We got a detailed picture about all their efforts to help people in need (homeless people, victims of catastrophe, etc.). I like their announced attitude: "Making a difference



Fig. 18. A wonderful Fulbright dinner in the house of our cultural referee

is not our job... it is our passion, our culture and our way of life". Another very interesting and remarkable excursion was *the visit to the Los Angeles Times and Downtown Los Angeles (Fig. 19, Fig. 20)*. We were guided through the main building, and a great many interesting aspects and background details were explained about journalism. It is really incredible how many happenings there are worldwide, and how the novel technologies and the media help the quick spreading of the news. As the John Wayne Cancer Institute is located

in Santa Monica, I only rarely had the opportunity to go downtown. Mrs. Ann Kerr made a wonderful guide, so I got the main impressions, learned about historical and political happenings, and saw the very old and very new parts of downtown Los Angeles. The skyscrapers and the old buildings and bazaars of Oliveira street stand shoulder to shoulder. To tell the truth, my first real acquaintance with downtown LA was a very special one: *my first marathon run was the famous XXII. Los Angeles Marathon Run, on March 4th, 2007,*



Fig. 19.
Our visit to Los Angeles Times



Fig. 20.
The Fulbrighters on the Los Angeles Downtown excursion

starting at Universal Studios in Hollywood and ending after a 42.6 km (26,2 miles) route with a finish line in downtown LA (Flower street and 5th street) (**Fig. 21**). The Los Angeles Marathon was a great experience, not only as it was my first marathon run, but also because it provided so many nice impressions about human effort, assistance, and the mutual pleasure of different people and nations. The LA Marathon is a good proof for the fact that *everybody who wants it strongly enough and who makes each next step forward with true effort and patience can reach their goal*. I keep some pictures in my mind: one is that of a professional runner, who suddenly turned back to a disabled runner and offered him water and chocolates, just to help him to keep on running. I made the run for my research, which is on developing a cure

for cancer, and wrote that note on the back of my shirt in red, white and green, the Hungarian national colors. There was another wonderful excursion organized for the Fulbright scholars into the Marine museum (**Fig. 22**), a visit to San Pedro, and a wonderful walking tour through a golf course down to the ocean (**Fig. 23**). This event was on a free Sunday in the middle of May, and after so much laboratory and computer work, I was simply delighted to feel the fresh air near the ocean. We all enjoyed it very much and also the nice pick-nick before noon and a great barbecue in the home of the organizers. I attended an interesting, pleasant evening organized by the *Fulbright Organization in Great LA*, and listened to the American Fulbrighters' experiences in different parts of the world. I was glad to be able

Fig. 21.
My first Marathon run
was in Los Angeles



Fig. 22.
The most charming memory of
the Marine Museum



Fig. 23.
The Fulbrighters
arrived to the
Pacific Ocean

to afford this “out of laboratory evening”, as I learned about a great many interesting cultural, political aspects and international collaboration possibilities. Although my *day-today life was always the same, I enjoyed it and had the proud little feeling* of going to work in the morning and coming back late at night from the institute. The bus ride was quite long from JWCI in Santa Monica to the place where I stayed in Los Angeles. I saw many interesting people, as well as many sad and cheerful situations, and I experienced a kind of humanitarian care, patience, and acceptance of the different. I was working in Santa Monica, one of the world’s famous holiday places, but I did not have too much leisure time to see all its beauty. As I never went out during the day, I saw just the morning

sun. I enjoyed the dawn only once, when coming out a bit earlier. But I roller skated along the wonderful Santa Monica Pier a few times and went jogging on Saturdays and Sundays from Beverly Hills to the Ocean, before my laboratory work. All these were great adventures. My supervisor and the colleagues at *the Department of Glycoimmunotherapy seemed to be a nice group of people, quite international and cheerful*. We celebrated birthdays with a lunch together and a movie. That was my first time seeing a movie in America, and I enjoyed these few occasions (**Fig. 24.**). I was glad to run in the Revlon Run/Walk for Women event together with colleagues at the JWCI, helping to fight women’s cancer this way, also (**Fig. 25.**). I will always keep the red JWCI T-shirt



Fig. 24.
Nice events in the Department of Glycoimmunotherapy



Fig. 25.
The Revlon Run in LA to fight against Women’s cancer

Fig. 26.
The „wandering circus” flies back home.



and the memories of a wonderful Saturday morning arriving to the LA sports arena together with all those others fighting against cancer in different ways.

4. Extension Period With Achievements And Difficulties

Because of the extension process and for important scientific reasons, and due to institutional and personal obligations in Hungary, *I had to make a trip to Hungary during the summer*. I was invited to give a scientific lecture at the international ISIR meeting in Croatia. As I have been an active member in this immunological society and a project leader on a special related topic, I was invited to give a *lecture and present a poster* about my research related to novel immunological diagnostics. The congress provided high impact knowledge on tumor-immunological problems and novel

immuno-therapeutic approaches. I also gave a lecture at the Institute of Oncology in Budapest. I took the chance while there to analyze by confocal laser microscopy my slides, previously stained with my purified antibody fragment. Parallel to that, *I had the opportunity to make some further DNA samples* to serve the melanoma interest at the JWCI group. I made quite a few *immuno-histological stainings on the tissue sections* obtained from the cancerous tissue samples of interest. All this took some time, but it was essentially helpful so as to continue my actual experimental works at JWCI. Meanwhile, I was informed that my previous Hungarian institute, the National Medical Center, had closed down. I thought I might continue my research work at JWCI (with the granted extension). But when I arrived back to JWCI, I realized that everything had changed quite unexpectedly. I could hardly talk to anybody, neither to my



Fig. 27. My sample preparations arrived home safely and frozen!

supervisor, nor to the head of the institute. *As a result of serious institutional changes, the Department of Glycoimmunotherapy was completely reorganized:* there was a different department head, there was no access to the laboratories we had previously equipped with my supervisor, and I could not communicate with former colleagues or with the supervisor. There were completely different circumstances and no information or explanations about how the original project with my preparations would continue. I had to work out new plans and possibilities, to determine how the great amount of biological preparations might be shipped back in suitable deep-frozen condition, once the Fulbright period would be over. Because of these drastic institutional changes I had to ask the help of CIES and the Fulbright Commission for negotiations, to be able to obtain and pack for shipment my preparations, essentially important for all the subsequent experimental work. I had plenty of results and achievements during my Fulbright period, as I had detailed earlier. I just felt sorry, as I really liked the original Department of Glycoimmunotherapy at JWCI, I highly appreciated the knowledge of my supervisor, and I had good impressions of the institute. *However, I knew that I would find my way again to continue the whole huge project and guide it to its diagnostic and therapeutic goal.* My “wandering circus” moved on, back to Budapest (**Fig. 26.**). I think no one else felt happier than I when, after a lot of nerve-racking arrangements and the indispensable help of my sister in Budapest, my biological preparations arrived home safely (**Fig. 27.**).

5. Epilogue

5.1. Summary And Conclusions Of The Work

The proposal's aim was to explore a very new area in tumor-immunology, leading to the development of tumor-specific antibodies-based immunoconjugates with potential diagnostic and therapeutic usage. A great arsenal of cellular, immunological, molecular, genetic, and biotechnological methods were necessary for the project. The John Wayne Cancer Institute focused on therapeutic antibody and vaccine development and the Department of Glycoimmunotherapy was highly relevant because it had a great background in developing the potential of gangliosides in cancer therapeutics.

* The results of the methodological process provided a purified antibody fragment, specific to unique tumor-associated gangliosides. This product is suitable for the development of an in vitro and/or in vivo tumor diagnostic reagent.

* There is a good chance that the special characteristics of this antibody can be used for an effective targeted tumor immunotherapy, because it has several advantages in comparison with the available conventional methods.

* The successful immunoglobulin repertoire analysis of tumor infiltrating B cells in further breast carcinomas and melanomas helps to clarify highly important tumor-immunological questions, concerning tumor-specific antigens.

The powerful technique I developed and used in breast carcinomas and melanomas was progressing. The main importance of

the work is that tumor-specific antibodies of human origin can be obtained this way! This is a far better solution for tumor therapy than the trials with murine monoclonal, humanized and chimeric antibodies. As gangliosides are centrally involved in the tumor progression, the anti ganglioside antibodies of human origin have a very good chance of being developed into effective diagnostic and therapeutic tools.

5.2. And The Work Goes On

Thank God, another new door has opened, through a possibility *at the Institute of Oncology in Budapest, at the Department of Tumorprogression (Fig. 28.)*. *The work goes on!* And the international *Hungarian – American scientific bridge built through the Fulbright Grant possibility remains*. When writing this report, I have just returned from a scientific trip to the US: I was invited to give a lecture at the NIH Clinical Center/ Bethesda, MD/ Washington, DC, and we discussed the further scientific collaboration work

with the interested department head and my Fulbright supervisor (Dr. Mepur H. Ravindranath, PhD) now at the Terasaki Foundation Laboratory/ Los Angeles. We keep the scientific communication with the former collaborative foundation (now: Integrated Medical Sciences Association Foundation) in San Diego also. *As soon as I have finished the novel data evaluation, the scientific papers will be written and published. The treasures of the Great Fulbright Experience are kept in my mind and heart.* I keep all my souvenirs in a real “treasure box”.

I am thankful for the great Fulbright experience and the Fulbright research grant (Fig. 29.).

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Fig. 28.
And the work goes on, through another possibility at the National Institute of Oncology, Budapest



Fig. 29.
I acknowledge the Fulbright Research Grant