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21 p. tables graphs

International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

Collaborative Research Programme

TERMINAL EVALUATION REPORT

UNIDO contract # 92/037

ICGEB ref. #: CRP/ ARG91-02

Project initiation: May 1992

Project termination: May 1995

B3/0

International Centre for Genetic Engineering and Biotechnology
United Nations Industrial Development Organization

Collaborative Research Programme

TERMINAL EVALUATION REPORT

Part 1

Title of Project	
"Humanization of Monoclonal Antibodies for Cancer Treatment"	
Keywords: Monoclonal Antibodies/Breast cancer/Melanoma/Humanization	
UNIDO contract # 92/037	**ICGEB ref. #:** CRP/ARG91-02
Project initiation: May 1992	**Project termination:** May 1995
Principal Investigator's name: Dr. José Mordoh	
Affiliate Centre mail address : Programa de Biotecnología Secretaría de Ciencia y Técnica Córdoba 831- Buenos Aires- Argentina	
Telephone no. 312-1706	**Telex no.**
Fax no.	**Email address**

Abstract:

The use in cancer therapy of MAbs directed against tumor associated antigens (Ags) offers the theoretical advantage of greater specificity than that obtained with conventional cytotoxic agents. However, most of the clinical results performed so far yielded only modest results (see i.e. Saleh M.N.et al. Cancer Res. 53: 4555-4562, 1993). A priori, the lack of success of MAbs in therapy could be due to several reasons: i) heterogeneity in Ag expression within tumors; ii) binding to inappropriate antigens; iii) shortage of effector mechanisms; iv) poor MAb permeation into tumors; iv) short interval of exposure of tumor cells due to the clearance kinetics of murine MAbs, and v) human anti-mouse immunoglobulin (HAMA) formation. One of the causes of cellular heterogeneity in tumors is the coexistance of cellular subpopulations with different grades of differentiation , i.e. stem cells, semi-differentiated cells and differentiated cells (see i.e. Ballare C. et al., Cancer 64, 842-848, 1989).

Since an aim of any curative intent is the elimination of the tumor stem cells, in our laboratory we have developed several mouse (m) monoclonal antibodies (MAbs) directed against tumor proliferating cells. Specially interesting is FC-2.15, since in the presence of complement it diminishes the clonogenic potential of tumor cells but not of normal bone marrow. An extensive description of it has been performed (1,2). Basically, FC-2.15 is an IgM obtained by immunizing Balb/c mice with tumor epithelial cells from a human undifferentiated carcinoma. Its main features are: 1) It reacts with more than 90 % of breast primary tumors, independently of their histology and hormone receptors content; 2) It reacts with 80 % of total breast malignant cells and with more than 90 % proliferating cells; 3) It recognizes other neoplasia such as colon cancer, squamous carcinoma and melanoma; 4) It mediates cellular lysis by complement and diminishes the growth rate of mammary tumors implanted in nude mice. The FC-2.15 has entered a Phase I Clinical Trial and in view of its clinical efficacy we achieved its humanization as well as we intended to obtain a greater variety of MAbs directed against the stem cell subpopulation.

Part 2

OBJECTIVES/METHODOLOGY
(proposed at the time of the submission of the research proposal)

The transformation of monoclonal antibodies (MABs) molecules of mouse origin into human will be carried out, as a first step, by substituting the mouse C regions of both chains by the human ones. The antigen binding specificity, carried by the V regions, will remain of mouse origin and thus the antigenic recognition sites should not be altered. The strategy that will be followed to obtain the chimaeric molecules consists of the use of recombinant DNA technology to construct a gene consisting of the V region with the sequence of the original murine MAB followed by the C region of human origin. This will be done with both the light and the heavy chains.

The work plan consists of:

1) To obtain the nucleotide sequence and the corresponding DNA fragments of the V-L and V-H regions. These sequences are of a lenght of around 300 bp and will be obtained by PCR amplification of the corresponding V sequences, using specific primers. In both cases the 3' primers (forward reactions) are homologous to 5'end of the C region. The 5'primers (reverse reactions) homologous to the 5'end of a consensus V region are synthesized with the appropriate degree of degeneration to cover for all different V regions. The fragments thus produced are then cloned and sequenced.

2) The amplified DNA fragments are then properly recloned upstream of the human Ck gene for the L chain and of the human Cg1 gene for the H chain. These chimaeric constructs are later cloned downstream of an eukaryotic strong promotor. Vectors already developed harboring the LTR promotor of the RSV and the murine Ig promotor and enhancer will be used to obtain the expression of both chains. These constructs carry also the selective marker gene for Neomycin resistance under the control of the HSV-TK gene promotor.

3) Two different cell lines will be used for the transfection of the chimaeric genes: CHO cells and the murine myeloma NSO (non-Ig producer). In both cases the cells will be co-transfected with the L and H chain genes and selected for resistance to the G418 antibiotic.

4) Individual clones of resistant transfectants will be isolated and assayed for their capacity to produce and secrete human Ig molecules. The specificity of the chimaeric MABs will be assayed using several criteria: a) adequate competition with the mouse MAB; b) similar range of reactivities against tumor tissues; c) similar capacity to mediate complement- and cell-mediated toxicities against tumor cells. The techniques that will be used for this purpose will be ELISA, immunohischemistry and immunocytochemistry.

In order to obtain high yields of secretion of the antibody, the transfection of the chimaeric genes will be also done cotransfecting a plasmid with DHFR gene in order to induce amplification of all transfected sequences by selection with methotrexate.

In a second step, more exquisite antibodies will be developed, based on the substitution of the framework sequences of the mouse V regions by those of human V genes in order to produce an Ig chain with the minimum of murine residues. In this way only the CRD regions of both H and L chain, which represent the main antigen binding specific residues, will remain of the original murine antibody.

RESULTS
(compare against the set objectives)

As it was described above the mMAb FC-2.15 is an IgM obtained by immunizing Balb/c mice with tumor epithelial cells from a human undifferentiated carcinoma. Its main features are: 1) It reacts with more than 90 % of breast primary tumors, independently of their histology and hormone receptors content ; 2) It reacts with 80 % of total breast malignant cells and with more than 90 % proliferating cells; 3) It recognizes other neoplasia such as colon cancer, squamous carcinoma and melanoma.

We could also observed:
1) By Western blots it detects three major bands of Mr 160, 130 and 115 kDa in membrane extracts of MCF-7 tumors grown in nude mice; 2) It has strong cross-reactivity with kidney proximal convolute tubules, large bowel epithelium, bone marrow myeloid progeny and peripheral granulocytes; 3) In view of the previous observation we investigated the reactivity of FC-2.15 with peripheral blood PMN leukocytes obtained from healthy donors. In our last report we informed several observations that allow us to demonstrate that the antigen recognized by our antibody is also present in high quantity in PMN leukocytes. Western Blots assays demonstrated that it is not the same band pattern than that detected in MCF-7 extracts. Three major bands of Mr 250 kDa, 185 kDa and 115 kDa were detected in PMN extracts. Since in these cells the antigen recognized by FC-2.15 seems to be in higher quantity than in MCF-7, we have purified the antigen from large amounts of PMNs in order to obtain enough material to perform its sequentiation.

FC-2.15 has shown clinical efficacy in Phase I clinical trials (3). Since the employ of this mMAb generated in patients human anti-mouse antibodies (HAMA), its humanization has been undertaken to circumvent this problem. For this purpose the initial step performed was the obtention of total RNA from the selected hybridoma. Afterwards, in Dr. Burrone's laboratory (ICGEB-Trieste) the cDNA fragments coresponding to the light (V-L) and heavy (V-H) variable regions from mouse FC-2.15 MAb were obtained by reverse transcriptase reaction. Once these fragments were amplified by polymerase chain reaction (PCR) techniques with oligonucleotides designed in Dr.Burrone's Laboratory, they were sequenced and cloned into suitable vectors which contain the mouse Fc regions replaced by human IgG_1 constant region.The chimaeric MAb obtained was expressed in eukaryotic systems and the IgG human-producing transfectants were selected. Several of them were obtained from murine myeloma and CHO cell lines.

Four main results were obtained with this process:

a) By comparing the nucleotide sequences of the corresponding V-L and V-H fragments of FC-2.15 mMAb in a Data Base, it could be seen that it was an original MAb since it had a very low homology with other mMAbs described.

b) Assays performed with ten-fold concentrated serum-free supernatant from some of these transfectant clones, showed that this chimaeric antibody recognizes by Western-blot assay, the same antigen than the murine FC-2.15 in PMN cells (Mr 250 kDa, 185 kDa and 115 kDa) (Figure 1).

c) It could be observed in ELISA assays that the chimaeric MAb retained the specificity against whole cells or extracts of MCF-7 cells, although the intensity of the positive signal was lower. This fact is probably due to the different isotype of the chimaeric MAb, since the mMAb was an IgM with more sites of antigen recognition. In spite of this positive result with ELISA assays, it was not possible to detect positivity in immunocytochemistry assays and biological activity showed no complement lysis in [Cr^{51}]assays. This result made so far unviable our project about the probably use of the humanized MAb in human therapy.

Part 3

Work plan and time schedule (originally envisaged)

The work plan that will be carried out involves several interelated projects :

1) Since we have already produced some mouse (m) MAbs (FC-2.5 and FC-2.6), that possess the ability to induce clinical responses in breast cancer patients their humanization will be started following the above outlined procedures. As a first step, the replacement of the mouse Ig'C regions will be performed. Once the chimaeric MAbs are obtained, their expression in eukaryotic systems will be attempted, and the selection of Ig - producing transfectants will be carried out. Once this step is achieved, the specificity of the MAbs will be analyzed by comparison with the original mMAbs.
2) Once the previous steps are satisfactorily completed, the chimaeric antibodies will be produced in a larger scale. After adequate purification and controls, their use in Phase I Clinical Protocols similar to those already being conducted would be considered. The main point at this step would be to determine if the chimaeric MAbs have lost the ability to induce a human immune response.
3) The construction of chimaeric MAb molecules only containing the mouse CDR regions will be attempted at a later stage, and a similar sequence of assays to those already described will be performed.
4) The antigens recognized by the MAbs FC-2.5 and FC-2.6 have yet to be characterized. The recognition and isolation of these antigens would enable us to obtain second-generation mMAbs that could have greater affinity and mediate more efficiently complement and cell - mediated toxicities.
5) The antigenic characteristics of the tumor stem cells will continue to be studied. Such knowledge would enable us to obtain a greater range of MAbs directed against the tumor stem cells. These new MAbs will have to be characterized and assayed for their activity against tumor cells. Those MAbs that prove more interesting could be also submitted to the humanization procedure.

TIME SCHEDULE

1st. year :
a) Humanization of MAbs FC-2.5 and FC-2.6.
b) Expression of chimaeric MAbs in eukaryotic systems.
c) Identification of the antigens recognized by MAbs FC-2.5 and FC-2.6.

2nd. year :
a) Large - scale-production of chimaeric MAbs.
b) Specificity determination.
c) Preparation of second-generation mMAbs.
d) Construction of chimaeric MAbs containing only mouse CDR regions.

3rd. year :
a) Humanization of second-generation MAbs.
b) Assay, alone or in combination, of chimaeric MAbs obtained, starting from in vitro to clinical assays.
c) Use of hMAbs in Phase I Clinical trials.
d) Obtention of new mMAbs directed against tumor stem cells.

Results (continue)

Actually in Dr.Burrone's laboratory the nucleotide sequences of the corresponding V-L and V-H fragments were subcloned in vectors containing the mouse constant region replaced by human IgM constant region. In that way we expect to obtain the same specificity reached with the mMAb which is also an IgM. However we must circumvent the current instability of the transfected clones and reach clones with high production (> 10 µg/ml).

d) We failed in the attempt to select a clone producing more than 0.6 µg/ml of the chimaeric immunoglobulin. Consequently, the purification procedures of the IgG_1 human - mouse immunoglobulin to obtain enough quantity for the subsequents experiments of biological activity, involved much more time and materials than expected.

However, we achieved several advances in our knowledge about the MAbs developed in our laboratory.

1) The antigenic profile of the tumor stem cells has been studied. Such knowledge will enable us to obtain more information about this subpopulation of tumor cells. Actually we could establish using a repertoire of mMAbs (COL-12; B1.1; B72.3; 4.36; MBr1 and FC-2.15) mainly directed against tumor associated antigens, an orderly pattern of antigen expression displayed by breast primary tumors which could be associated with their differentiation grade (4).These results confirmed again the usefulness of our MAb FC-2.15 in recognizing undifferentiated tumors.

2) We could establish a new human undifferentiated primary breast carcinoma cell line (5) (IIB-BR-G), which displays a low antigenic profile. This characteristic makes it useful to generate new murine MAbs, since this cell line displays a very agressive behaviour.
We have also established two new melanoma cell lines (6) (IIB-MEL-LES and IIB-MEL-IAN) which alowed us to analyze in more detail the melanoma behaviour.

3) Acording with the last point we studied in the three melanoma cell lines developed in our laboratory as well as fixed biopsies of primary and metastatic melanomas and non malignant nevi. A sequence of events leading from nevocellular nevi to dysplastic nevi to melanoma has been proposed in developing of this malignancy. We have demonstrated that the 52 kD aspartil protease Cathepsin D is highly expressed in primary and metastatic melanomas and the great majority of dysplastic nevi, whereas only 18 % of nevocellular nevi express this protease, and normal melanocytes express none. These data suggest that the expression of Cathepsin D might be associated with melanoma development (7).

4) New murine MAbs were obtained by immunizing Balb/c mice with the breast carcinoma cell line above mentioned (IIB-BR-G). One of these MAbs (FC-5.01) showed interesting characteristics. It is an IgG_{2a}, it recognizes an antigen expressed in membrane of breast cancer cells and of melanoma cells, which in permeabilized cells is found in intracytoplasmic vesicles. The antibody could be internalized. The above mentioned characteristics makes it useful to employ it in RID (Radio Immuno Detection) and in human therapy conjugated with toxins. This MAb in Western - Blot assays developed in native conditions, recognized a very high molecular weight band. The positive reactivity in Western or ELISA assays is lost when the sample is heated at 100°C (manuscript in preparation).

5) Concerning the clinical trial using the murine FC-2.15, a Phase I study was performed in 11 patients with advanced cancer (breast=4, colon=2, melanoma=2, lung=1, medullary thyroid=1, skin epidermoid carcinoma=1). MAb was administered by i.v. infusion every other day, with doses ranging between 2.5 and 5.0 mg/kg. Maximal FC-2.15 serum concentration ranged between 2.2 and 7.8 µg/ml, and its serum

Results (continue)

half-life was about 9 hr. All patients developed HAMA. The most consistent side effect (10/11 patients) was a profound and selective neutropenia which occured within 1 hr after the start of each infusion and reversed within 1 hr after its discontinuation. Other frequent side effects included fever < 38 °C and chills that were easily manageable. None of these effects required dose reduction or treatment interruption. A complete response of a basocellular carcinoma and a sustained (6 months) > 50% partial response of breast carcinoma liver metastases were observed.

6) A Phase II clinical trial has began with this mMAb, employing in this oportunity an immunosuppressive drug (deoxyspergualin), commonly used in renal transplants. In the few cases evaluated (n=6) a significative retard in HAMA development could be observed.

Work plan and time schedule

(actual)

Project landmarks, duration of individual tasks (use bar charts); evaluation criteria (publications, patents, services, training)

1) The original work plan involved the humanization of MAbs FC-2.5 and FC-2.6 (actually renamed FC-2.15 and FC-2.16). The sequentiation of the V region from both heavy and light chains of the aboved mentioned MAbs, revealed that they were basically two different clones producing the same protein, with only one different aminoacid residue. Consequently the subsequents steps were continued with one of the clones.
The chimaeric huFC-2.15 IgG obtained was analyzed by comparison with the original murine MAb. Although the previous steps were satisfactorily completed as judged by Western-blot analysis in which the huFC-2.15 recognized the same three bands in membrane extracts of PMN cells, it could not retain the biological activity. HuFC-2.15 IgG was not able to produce complement mediated lysis in [Cr^{51}] assays. This fact is probably due to the different isotype of the original MAb (IgM).
This result made so far unviable our project about the probable use of the humanized MAb in human therapy. Although in Dr.Burrone's laboratory these steps were modified to obtain in this opportunitty a huFC-2.15 IgM, this soon has to be evaluated.

2) The antigens recognized by the FC-2.15 were partially characterized since we could observe in Western-blot assays, three major bands of Mr 160, 130 and 115 kDa in membrane extracts of MCF-7 tumors grown in nude mice. The antigen recognized in PMN cells revealed also three bands (Mr 250 kDa, 185 kDa and 115 kDa). The sequentiation of the antigen obtained from extracts of large quantities of PMN cells is now achieved. It remains to establish if the antigens recognized by this MAb in colon carcinomas display the same pattern of breast cancer or PMN. We have obtained a human colon tumor growing in nude mice with the purpose of answering this question.

3)The antigenic characteristics of the tumor stem cells were further studied. This knowledge could enable us to obtain a greater range of MAbs directed against tumor cells. One of this, FC-5.01, obtained through the immunization of BalbC mice with a poorly undifferentiated human breast cancer cell line established in our laboratory, showed interesting characteristics as it was mentioned in Results. We could also establish an orderly pattern of expression displayed by breast primary tumors which could be associated with their differentiation grade.

Therefore, in spite of the above mentioned difficulties, we achieved several advances in our knowledge about the MAbs developed in our laboratory, and a more detailed description of them appeared in "Results".

NETWORKING

NETWORKING : This Project is being carried out in collaboration with **Oscar Burrone, Ph.D.**, Head, Molecular Immunology Group, International Centre for Genetic Engineering and Biotechnology.

PUBLICATIONS

Work plan (actual) (continue)

Duration of individual tasks:

Name	Role in Project	1° year	2° year	3° year
Mordoh, José. M.D.	Principal Investigator	———	———	———
Barrio, Marcela. M.Sc.	Assistant Investigator (Cellular Biology)	———	———	———
Bravo, Ines M.D.	Associate Investigator (Pathologist)	———	———	———
Kairiyama, Claudia. M.Sc.	Associate Investigator (Biochemistry, Immunology)	———	———	———
Leis, Silvia. M.Sc.	Assistant Investigator (Immunology)	——		
Podhajcer, Osvaldo. Ph.D.	Associate Investigator (Molecular Biology)	———	———	———
Bover, Laura. Ph.D.	Associate Investigator (Cellular and Molecular Biology)		———	———

Training

During these 3 years, the people involved in this project adquired considerable new experience in Immunology techniques and in Molecular Biology.

PUBLICATIONS

1) "Description of a new monoclonal antibody, FC - 2.15, reactive with human breast cancer and other human neoplasia".
J.Mordoh, S.Leis, A.I.Bravo, O.L.Podhajcer, C.Ballaré, M.Capurro, C.Kairiyama y L.Bover.
American Association for Cancer Research Annual Meeting. San Francisco, U.S.A. 1994.

2) "A new monoclonal antibody, FC-2.15, reacting with human breast cancer and other human neoplasia".
J.Mordoh, S.Leis, A.I.Bravo, O.L Podhajcer, C.Ballaré, M Capurro, C.Kairiyama y L.Bover.
Int.J.Biol. Markers 9: 125-134. 1994.

Work plan (actual) (continue)

3) "A Phase I clinical trial in cancer patients of a new monoclonal antibody, FC-2-15, reacting with tumor proliferating cells".
Mordoh, J., Silva, C., Albarellos, M., Bravo, A.I. y Kairiyama, C.
J.Immunotherapy 17(3): 151-160, 1995.

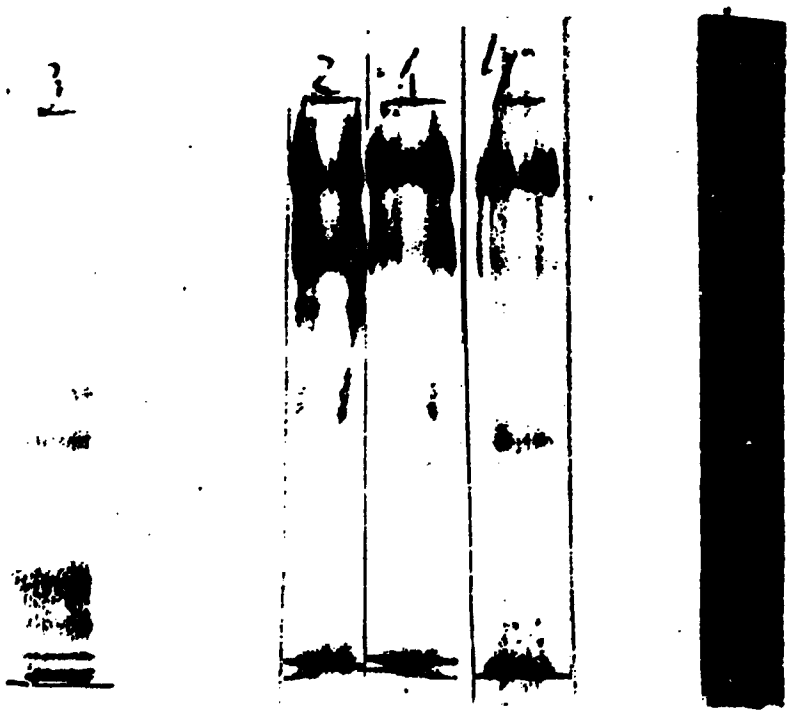
4) "Marker expression and differentiation in human breast cancer".
Ballare C., Bravo A.I., Turchi V., Nuti M., Yomha R., Schiaffi J. and Mordoh J.
Ann.NY Acad.Sci. 698: 143-147, 1993.

5) "Description of a new human breast cancer cell line, IIB-BR-G, established from a primary undifferentiated tumor".
L.Bover, M.Barrio, I.Slavutsky, A.I.Bravo, C.Quintans, A.Bagnati, B.Lema, J.Schiaffi, R.Yomha yJ.Mordoh.
Breast Cancer Res.Treat. 19: 47-56, 1991.

6) "Biologic, immunocytochemical and cytogenetic characterization of two new human melanoma cell lines: IIB-MEL-LES and IIB-MEL-IAN".
C.Kairiyama, I.Slavutsky, I.Larripa, V.Morvillo, A I.Bravo, L.Bover, O.L.Podhajcer y J.Mordoh.
Pigment Cell Res (in press).

7) "Characterization of cathepsin D expression in human malignant melanoma, dysplastic nevi and nevocellular nevi".
O.Podhajcer, L.Bover, A.I.Bravo, M.F.Ledda, C.Kairiyama, I.Calb, L.Guerra, F.Capony y J.Mordoh.
J.Invest.Dermatol. 104: 340-344,1995.

8) "Functional properties of FC-2.15, a monoclonal antibody that mediates human complement cytotoxicity against breast cancer cells.
Ballare, C., Barrio, M., Portela, P. y Mordoh, J.
Cancer Immunol. Immunother. (in press).


3
2
1
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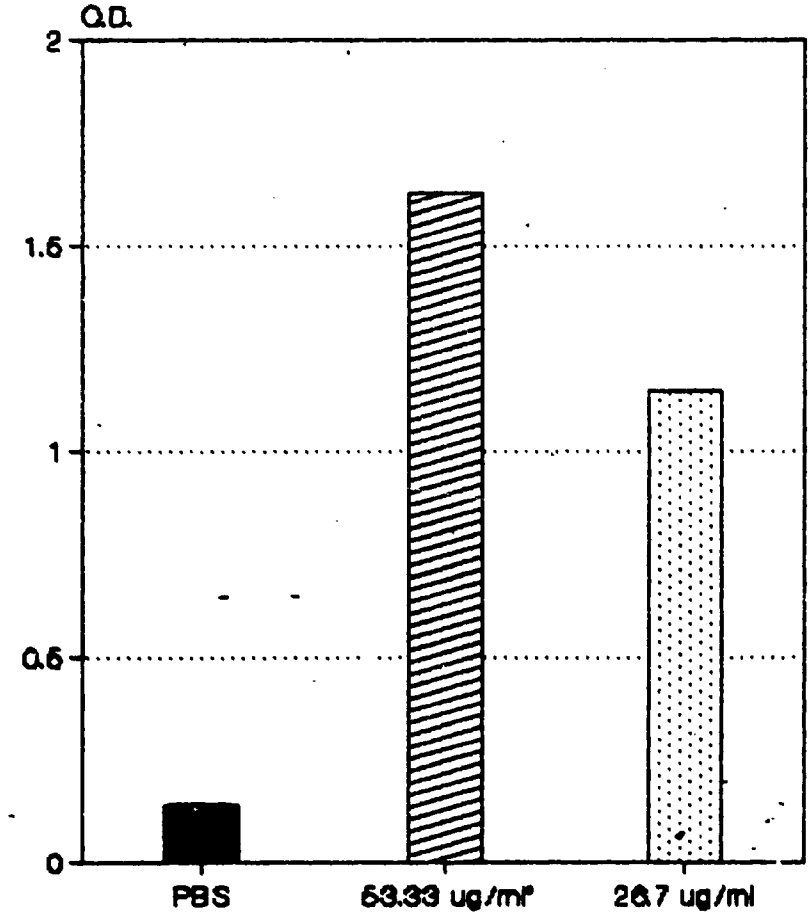
ELISA assay of purified huFC-2.15 IgG
Hybridoma T1SG7-3D6 Clon 22

Figure 2



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Part 4

STATEMENT OF EXPENDITURES

To be filled by ICGEB		To be filled by the Affiliated Centre	
Budgets as per original proposal		**Summary of expenditures** *	
1) Capital equipment	US$	1) Capital equipment	US$ -
2) consumables	US$	2) consumables	US$ 9,345.68
3) training	US$	3) training	US$ 3,900.00
4) literature	US$	4) literature	US$ -
5) miscellaneous	US$	5) miscellaneous	US$ 6,755,03
TOTAL GRANT	US$.....	**TOTAL**	US$ 20,000.71

Please itemize the following budget categories (if applicable)

Capital equipment

Training (provide names, duration of training, host laboratory)

PAULA PORTELA - 6 months - Laboratory of Cancerology-Fundación Campomar

Literature

* Please do not send invoices, receipts etc.; these should be kept by the Affiliated Centre for future reference and sent to ICGEB upon request.

Director: MORDOH, José
Legajo: Con.92/037
Instituto/Tema: UNIDO PROJECT No. GE/GLO/89/002 Sigla:

Fondos otorgados por **UNIDO** RUBRO **CONSUMABLES**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto U$S
1	05/01/94	Pharmacy	Tryosom and penicillin		62,60
2	05/01/94	Farmacia S.Eduardo	Gauze		3,00
3	14/02/94	Migliore-Laclaustr	125 Iodine and 51 chromium		335.00
4	03/03/94	Central de Palermo	Gauze		7,50
5	08/03/94	Química Elfand	Ethanol		19,50
6	16/03/94	Norcopi	Paper and labels		24,90
7	24/03/94	Maxi Kiosco	Kodak film		7,50
8	30/03/94	Photo Farben	Film		10,00
9	30/03/94	Neutro Química	Corning bottles		34,00
10	30/03/94	Neutro Química	Bovine fetal serum		960,00
11	20/04/94	Neutro Química	Corning filters and bottles		330.00
12	20/04/94	Neutro Química	Restriction enzymes		170,00
13	25/04/94	Química Elfand	Neubauer chamber		84.00
14	06/05/94	Trowells	Petri dishes, bottles, cuvettes and cryotubes		349,00
15	06/05/94	Farmacia Electra	Syringes		3.00
16	06/05/94	Neutro Química	Petri dishes, bovine fetal serum, Corning bottles and centrifuge tubes		364.00
17	11/05/94	Farmacia Rex	Medicine		50.00
18	17/05/94	Trowells	Corning pipets		150.00
19	19/05/94	Farm.Centenario	Heparin		10,64
20	23/05/94	Migliore-Laclaustr	125-Iodine and 51-chromium		326,00
21	02/06/94	Izaguirre	Rabbit		14.00
22	07/06/94	Neutro Química	Corning and Falcon bottles and bovine fetal serum		443,00
			SUBTOTAL:	0,00	3.857,64

Firma responsable:

Director: MORDOH, José
Legajo: Con.92/037
Instituto/Tema: UNIDO PROJECT No. GE/GLO/89/002 Sigla:

Fondos otorgados por **UNIDO** RUBRO **CONSUMABLES**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto U$S
			Subtotal.....	0,00	3.857,84
23	07/06/94	MAB	Tubes		148,00
24	07/06/94	Neutro Quimica	Petri dishes and Corning bottles		140,00
25	08/06/94	Mei-Lin	Kodak film		6,50
26	16/06/94	Mei-Lin	Kodak film		6,50
27	27/06/94	Migliore-Laclaustr	125-Iodine		168,00
28	30/06/94	AP Internacional	Filters		292,00
29	01/07/94	Schueler & Co.	Teflon combs and card		46,30
30	01/07/94	Neutro Quimica	Falcon and Corning bottles, bovine fetal serum and pipets		[illegible]
31	01/07/94	[illegible]	Corning Petri dishes, cryotubes and bottles		132,00
32	11/07/94	Farmacia Electra	Syringes		3,00
33	01/08/94	Migliore-Laclaustr	35S-Methionine and 125-Iodine		404,00
34	01/08/94	Química Elfand	Ethanol, methanol, blades and syringes		235,40
35	04/08/94	Izaguirre	Milk		31,00
36	08/08/94	Farmacia Electra	Heparin		3,00
37	19/08/94	Jablonka	Polaroid film		55,76
38	29/08/94	Migliore-Laclaustr	3H-Thymidine		240,00
39	01/09/94	Neutro Quimica	Culture medium, Corning and Falcon bottles and bovine fetal serum		493,00
40	08/09/94	Farmacia Electra	Penicillin and streptomycin		49,30
41	14/09/94	Farmacia Electra	Syringes		4,50
42	19/09/94	Migliore-Laclaustr	51-Chromium and 125-Iodine		300,00
			SUBTOTAL:	0,00	7.129,40

Firma responsable:

Director: MORDOH, José
Legajo: Con.92/037
Instituto/Tema: UNIDO PROJECT No. GE/GLO/89/002 Sigla:

Fondos otorgados por **UNIDO** RUBRO **CONSUMABLES**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto U$S
			Subtotal.....	0,00	7.129.40
43	21/09/94	Farm.Centenario	Syringes		14,00
44	22/09/94	Chemit	Formic aldehyde		17,20
45	27/09/94	Neutro Química	Corning cryotubes		70,00
46	27/09/94	Pharmacy	Roferon		58,04
47	27/09/94	Neutro Química	Bovine fetal serum		300,00
48	07/10/94	Valot SA	Tissue paper		68,40
49	11/10/94	Biopore	Filtering membranes		410.64
50	14/10/94	Neutro Química	Fungizone, bottles, petri dishes, bovine fetal serum, centrifuge tubes, buffer and sol/ cuvettes		751,[illegible]
51	17/10/94	Química Elfand	Syringes with needles		167.00
52	18/10/94	Neutro Química	Bovine fetal serum and petri dishes		360,00

TOTAL: 0,00 9.343,68

Director: MORDOH, Jose
Legajo: Con.92/037
Instituto/Tema: UNIDO PROJECT No. GE/GLO/89/002 Sigla:

Fondos otorgados por **UNIDO** RUBRO **TRAINING**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto US$
1	31/01/94	Paula Portela	Fellowship-january 94		600,00
2	28/02/94	Paula Portela	Fellowship-february 94		600,00
3	30/03/94	Paula Portela	Fellowship-march 94		600,00
4	29/04/94	Paula Portela	Fellowship-april 94		600,00
5	31/05/94	Paula Portela	Fellowship-may 94		600,00
6	30/06/94	Paula Portela	Fellowhip-june 94		900,00

TOTAL: 0,00 3.900,00

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Legajo: Con.92/037
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Fondos otorgados por **UNIDO** RUBRO **MISCELLANEOUS**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto US$
1	03/01/94	L.Millara	Photographic work		114,9[illegible]
2	12/01/94	Asoc.Banco Células	1994 Membership dues		20,00
3	14/01/94	Copifil	Photocopies		1.60
4	31/01/94	L.Millara	Photographic work		61,15
5	31/01/94	Virginia Adagini	Technical assistance		250,00
6	31/01/94	Rita Olivares	Glassware sterilisation		114,66
7	02/02/94	Fletes Kach	Mice and gas tubes transport		84,00
8	28/02/94	L.Millara	Photographic work		24,80
9	28/02/94	Rita Olivares	Glassware sterilisation		114,66
10	28/02/94	Virginia Adagini	Technical assistance		250,00
11	02/03/94	Fletes Kach	Liquid nitrogen and gas tubes transport		102,00
12	30/03/94	Virginia Adagini	Technical assistance		250,00
13	30/03/94	Rita Olivares	Glassware sterilisation		114,66
14	02/04/94	Fletes Kach	Liquid nitrogen and gas tubes transport		96,00
15	04/04/94	L.Millara	Photographic work		107,55
16	05/04/94	Copifil	Photocopies		70,00
17	05/04/94	Norcopi	Paper and roller pen		20,50
18	09/04/94	Photo Farben	Photographic work		19,00
19	29/04/94	Photo Farben	Photographic work		14,00
20	29/04/94	Rita Olivares	Glassware sterilisation		114,66
21	29/04/94	Virginia Adagini	Technical assistance		250,00
22	02/05/94	L.Millara	Photographic work		129,55
23	02/05/94	Fletes Kach	Gas tubes and mice transport		156,00
			SUBTOTAL:	0,00	2.477,77

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Fondos otorgados por **UNIDO** RUBRO **MISCELLANEOUS**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto U$S
			Subtotal.....	0,00	3.479,77
24	05/05/94	Photo Farben	Photographic work		12,00
25	14/05/94	Info Master	Power cable and covers		27,00
26	24/05/94	Photo Farben	Photographic work		10,00
27	27/05/94	Photo Farben	Photographic work		5,00
28	31/05/94	Rita Olivares	Glassware sterilisation		114,66
29	31/05/94	Virginia Adagini	Technical assistance		250,00
30	01/06/94	L.Millara	Photographic work		98,35
31	02/06/94	Photo Farben	Photographic work		10,00
32	02/06/94	Fletes Kach	Liquid nitrogen and gas tubes transport		156,00
33	07/06/94	OCA	Postage		3,50
34	30/06/94	Rita Olivares	Glassware sterilisation		171,99
35	30/06/94	Virginia Adagini	Technical assistance		375,00
36	02/07/94	Fletes Kach	Mice transport		90,00
37	05/07/94	L.Millara	Photographic work		304,40
38	05/07/94	Academia Nac.Medic	Bibliographic search		10,00
39	08/07/94	Academic Nac.Medic	Photocopies		0,60
40	28/07/94	Academic Nac.Medic	Photocopies		2,00
41	31/07/94	Rita Olivares	Glassware sterilisation		114,66
42	31/07/94	Virginia Adagini	Technical assistance		250,00
43	01/08/94	L.Millara	Photographic work		246,35
44	08/08/94	Photo Farben	Photographic work		23,00
45	11/08/94	Fund.Ejercito Arg.	Cell irradiation		300,00
46	18/08/94	Librería Indice	Photocopies		9,63
			SUBTOTAL:	0,00	5.063,91

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Fondos otorgados por **UNIDO** RUBRO **MISCELLANEOUS**

Nro.	Fecha	Referencia	Descripción	Monto $	Monto U$S
			Subtotal.....	0,00	5.063,9[illegible]
47	22/08/94	Fletes Kach	Mice transport		144,00
48	31/08/94	L.Millara	Photographic work		141,60
49	31/08/94	Rita Olivares	Glassware sterilisation		114,66
50	31/08/94	Virginia Adagini	Technical assistance		250,00
51	16/09/94	Fletes Kach	Mice transport		150,00
52	30/09/94	Rita Olivares	Glassware sterilisation		114,66
53	03/10/94	L.Millara	Photographic work		241,40
54	04/10/94	J.Mordoh/S.Adris	Taxi to Military Hospital - transport of irradiated cells		38,00
55	07/10/94	Maria M.Barrio	Taxi (transport of research material from several instit..		71,00
56	17/10/94	Laura Bover	Taxi (attendance course on Gene Therapy)		30,00
57	31/10/94	L.Millara	Photographic work		119,80
58	14/11/94	SAIB	1994 Membership dues		30,00
59	19/12/94	Fletes Kach	Liquid nitrogen and gas tubes transport		156,00

TOTAL: 0,00 6.755,00