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NEOPLASTIC TRANSFORMATION OF HUMAN CELLS

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The goal of this project was to gain a better understanding of the cellular mechanisms of cancer induction by ionizing radiation as a risk assessment for workers subjected to high LET irradiation such as that found in space. The following ions were used for irradiation: Iron, Argon, Neon, and Lanthanum. Two tests: growth in low serum and growth in agar were used as indicators of cell transformation. The specific aims of this project were: I. To compare the effectiveness of various ions on degree of transformation of a single dose of the same RBE. II. To determine if successive irradiations with the same ion (Fe 600 MeV/u) increases the degree of transformation. III. To test if clones with the greatest degree of transformation produce tumors in nude mice. IV. To construct a cell hybrid of a transformed and control (non-transformed) clone.

The cells used for this work are human mammary epithelial cells with an extended lifespan and selected for growth in MEM + 10% serum. A description of all cells used can be found in Appendix I. Over a 5 year period, cells were irradiated either with a single or multiple dose of the same ion or were irradiated several times by different heavy ions and later clones of various stages of transformation were frozen down resulting in a collection of single, multiple and mixed ion irradiated cells that were analyzed to check the effect of heavy ions on transformation. For details, see Appendix I and Appendix II. Selected clones were evaluated for growth in low serum media and formation of colonies in agar.

When comparing results from the two tests, growth in low serum and growth in agar, they do not correlate well. Cells that grow well in low serum do not necessarily form colonies in agar and vice versa. Overall, more clones grew in low serum. It appears that growth in agar measures a different aspect of transformation than growth in low serum. In comparing the relative effectiveness of the varying ions using a single dose, all ions were more effective than Fe in causing increased survival in low serum. Details of results are given in Appendix II. The effect of successive irradiations of iron did, in general, cause cells to become more transformed. Survival in 1% serum increased with passage number. Clones that had progressed to a more advanced stage of transformation did show increased survival in both 1% serum and agar. However, upon further irradiation, the survival tended to decrease for both tests. Clones that grew well in low serum and in agar were selected to test for tumor formation in nude mice. Out of 5 clones tested, one did form tumors.

We were successful in constructing a cell hybrid of control and transformed clones (Appendix I). This hybrid will be tested for growth in low serum and agar to see if transformation follows a dominant or recessive trait. We have since been able to identify a tumorigenic clone and isolate cells from tumors. We will fuse these tumorigenic cells to a control clone and examine this hybrid.

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CELL LINES USED

The cells used for this work are human mammary epithelial cells originally derived from a reduction mammoplasty tissue (specimens 184), with no apparent epithelial cell pathology. After treatment of 184 cells with benzo(a)pyrene, 2 clones with extended lifespan (184B5, and 184A1) were isolated[1]. Both clones are near diploid, each with a set of clonal chromosomal markers. They are non-tumorigenic in nude mice and show no anchorage independent growth. We irradiated the 184B5 cells with 200 rads of Iron ions (600 Mev/u) to produce media variants; i.e., cells that are able to grow in MEM +10% serum, and later BME + 5% serum, instead of the more complex serum-free medium MCDB 170 (filled with many necessary chemicals, hormones and drugs) required by the 184B5 cells.

Over a period of 5 years, some Media Variants[2] were irradiated either with a single or multiple dose of the same ion, others were irradiated several times by different heavy ions and later clones of various stages of transformation such as opaque colonies and agar colonies were isolated, resulting in a collection of single, multiple and mixed ion irradiated cells that were analyzed to check the effect of heavy ions on transformation.

Two cell lines were used as positive controls when testing growth in agar and tumor formation in nude mice: 1) A human carcinoma (HTB126 from ATCC) grew very well in 0.3% agar and was used until several irradiated Media Variant clones were found that grew equally well in agar. 2) A malignant 184A1 clone was used as a positive tumor control in nude mice. This malignant clone was produced by exposing 184A1 cells to Harvey sarcoma virus, and SV40-T antigen. These cells are highly malignant, show anchorage independent growth, and form tumors in 10/10 nude mice.

[1] Stampfer and Bartley, PNAS 82, 2394 (1985)

[2] For a description of the various Media Variants, see Section 2

Section 2A

DESCRIPTION OF 3 MEDIA VARIANTS: M10, M5, & M4

CLONE M10

On 1/14/88, 5-25cm2 flasks of 184B5 at passage 50, were irradiated with doses ranging from 40 to 200 rads Fe (600MeV/u). Initial irradiation was done in media MCDB 170. Shortly after irradiation, the cells from each dose were split into 5 flasks per dose to look for transformation. All cells were fed with MCDB 170 for 1 week after irradiation. Only the 200r flask gave cells that survived (flask F5). On 1/21/88, the cells were split 1:2. One set was fed with MCDB 170, the second set was fed with media MEM + 10% sera. The cells grew very slowly and the existing population was reduced to just a few cells. Out of the 5 flasks orig plated on 1/21, and fed with MEM, only 3 gave cells that survived: F5-1, F5-3 and F5-5. Within 1 month, on 2/19, the cells were again split but the population was still small and they continued to grow very slowly. Gradually, the population grew and cells from the 3 flasks were split 2 months later on 4/29. Growth rate was slowly improving so that on 5/17 the cells were again split 1:2 into 2 sets of flasks. Half of the flasks were fed with 10% sera, half with 5% sera. Eventually cells from flask F5-3 and F5-5 died out leaving only flask F5-1 with continuing viable cells. These cells were first frozen down on 12/22/88 almost 1 year after the initial irradiation. These cells have always been kept separate and never mixed with any of the other clones.

This M10 clone has shown the most consistent growth parameters through the past 7 years of culture: constant growth rate and %P.E., exhibits contact inhibition and anchorage dependence. The M10 clone has also shown that it is the most resistant to transformation from ionizing radiation requiring more irradiation to initiate transformation changes than the other series.

CLONE M5

This clone is derived from the M10 series and is composed of cells that could survive in lower calf sera. As described above, about 4 months after the initial irradiation, 3 flasks from the orig M10 clone were plated into MEM + 5% sera. This was the 5th passage of the originally irradiated cells. At passage 9, flask F5-5 was discarded because cells were not growing well. Although both flasks F5-1 and F5-3 gave cells that grew, F5-3 cells grew more slowly and was used for only one successive irradiation experiment. By far, the more healthy cells were in F5-1 and that clone was used for all other successive irradiations. Shortly after the 5/17 passage, a portion of cells were fed with 2% sera but the cells did not grow well and 5% was continued. One month later, on 6/17 the cells were exposed to 4% sera but again, they did not grow well, and were returned to 5% sera media.

The M5 clone is somewhat unstable and on one occasion an early passage media variant exhibited growth in agar. This could have been due to improper freezing conditions as it only happened once, and occurred immediately after being thawed out, however it does indicate a possibly unstable genome. Indeed, the only clone to produce tumors in nude mice was a M5 media variant that had been additionally irradiated only once with Iron ions.

CLONE M4

The M4 clone originated from a M5 clone in flask F5-1. During a routine passage of a M5 clone on 1/19/89, cells in flask F5-1 looked long and thin. As this was a possible morphological change toward transformation, these cells were passaged and kept separate from the other clones. This change was noticed at passage 11 and first separation started with passage 12. Although initially fed with 4% sera the cells grew better in 5% and were mostly kept in MEM + 5%. The designation M4 came from the early tests in 4%.

The M4 clone although more stable that the M5 clone is still not as stable as M10. Opaque colonies were observed and cloned from the original media variant at passage 19 however, that same passage did not produce colonies in agar. The M4 clone has been used for many successive irradiations and although it produced one of the most rapidly growing agar colonies clone (A11), it did not form tumors in nude mice.

Section 2B

DESCRIPTION OF CLONE I.D. AND NOTATION

Clones were first organized into a filing system based on the original Media Variant clone (M10, M5, M4), type ion irradiated, and number of times irradiated. Because many clones were already a product of an advanced transformation stage (i.e. opaque colonies, or agar clones) a particular notation was devised to describe this later selection, including passage number at each irradiation and transformation stage. In this way, a trend, if there was one, could more easily be seen.

I will give a short description of the identification system used. There are 2 parts to the identification of each clone: a clone number, and a notation. The clone number I.D. identifies and separates each clone by irradiation. Each time a clone was irradiated, it was given a different number. The notation describes the passage number at which the clone was irradiated or cloned. Later when these clones exhibited signs of transformation (presence of opaque colonies and growth of agar colonies), and further cloning of these colonies were done, the notation showing passage number and type of colony was indicated to reflect the new change. So the notation continued to change with each colony as it was passaged, but unless there was further irradiation, the clone number always remained the same. Only if that cloned opaque colony or agar colony was further irradiated did the clone number change. For example, on page 2 of Table I (Appendex II), the clone with the number ID 24 is a M4 Media Variant that received one dose of Fe. From this clone, one opaque colony and 3 agar colonies were later cloned, but they all still carry the same ID of 24. The description of the later clones will be indicated in the notation as to the passage at which each was cloned. Later, the opaque colony was itself irradiated with Fe, and it received a new number of 40 (page 3), and still later it was also irradiated with Ar and again received a new number of 61 (page 4). The original notation of clone 24 is F5-1/32(M4).Fe, indicating that the M4 Media Variant from flask F5-1 was irradiated with iron at passage 32. When the opaque colony was cloned, it had the notation of F5-1/32(M4).Fe/6.OC1 indicating that 6 passages after the iron irradiation, an opaque colony (#1) was cloned. After the 2nd irradiation, the new clone #40 had a notation of F5-1/32(M4).Fe/6.OC1/6.Fe indicating that at the 6th passage of the opaque colony it was irradiated with iron.

Section 3

PROCEDURES TO TEST TUMOR FORMATION IN NUDE MICE - CELL PREPARATION AND METHOD OF INJECTION

I. CELL PREPARATION FOR INJECTION INTO NUDE MICE

(PROCEDURE USED BY DR. PAUL YASWEN TO INJECT AIN4-TH CELLS INTO NUDE MICE)

1. TRYPSINIZE CELLS, AND SPIN DOWN, REMOVE SUPERNATE

2. RESUSPEND CELLS IN PBS

3. TAKE AN ALIQUOT FOR CFA

4. SPIN DOWN AND RESUSPEND IN A FINAL VOLUME OF PBS TO GIVE 10^7 CELLS IN 0.2CC

5. KEEP CELLS ON ICE UNTIL INJECTED INTO MICE

II. INJECTION OF CELLS INTO NUDE MICE

(COURTESY OF BIRGETTA KULLGREN)

1. NO. CELLS INJECTED: 10^7 CELLS IN 0.2CC PBS PER INJECTION SITE

2. LOCATION OF INJECTIONS: FAT PAD (WHERE SIMILAR/COMPATIBLE CELLS EXIST IN THE MOUSE)

3. USE A SEALABLE CONTAINER. A GLASS JAR IS PREFERABLE AS YOU CAN MONITOR THE MICE. TAPE COTTON OR GAUZE TO THE LID AND BOTTOM OF THE JAR. ADD ABOUT 1/2 TEASPOON METAPHANE[A] EACH TO THE GAUZE

4. PLACE MOUSE TO BE INJECTED INTO JAR AND SEAL LID.

5. MEANWHILE, PREPARE INJECTION USING A 1CC DISPOSABLE SYRINGE AND 25G DISPOSABLE NEEDLE. WITHDRAW 0.2CC CELLS AND HOLDING THE SYRINGE UPRIGHT, GENTLY TAP THE SIDES TO REMOVE ANY AIR BUBBLES.

6. MONITOR MOUSE CONSTANTLY. BREATHING SHOULD REMAIN CONSTANT IF BREATHING BECOMES IRREGULAR AND GASPING REMOVE MOUSE IMMEDIATELY AND ALLOW TO RECOVER (ABOUT 5-15 MIN.).

7. REMOVE MOUSE . PLACE MOUSE ON BACK WITH VENTRAL SIDE UP.

8. USING FOREFINGER AND THUMB, GENTLY PULL SKIN UP ABOUT 1/2 - 1CM FROM BODY. SLOWLY INSERT THE NEEDLE INTO THE OPEN AREA OF THE SKIN BEING CAREFUL NOT TO PUNCTURE THROUGH TO THE OTHER SIDE. YOU CAN MONITOR THE NEEDLE WITH YOUR FINGERS. BE SURE THE NEEDLE IS IN AT LEAST 1/2 CM. VERY SLOWLY BEGIN INJECTING THE CELLS WATCH CAREFULLY TO BE SURE THE LIQUID IS STAYING INSIDE. THIS IS A SUBCUTANEOUS INJECTION, SO YOU WANT THE CELLS CLOSE TO THE SURFACE BUT UNDERNEATH THE SKIN.

9. SLOWLY REMOVE THE NEEDLE AND CHECK THAT THE LIQUID DOES NOT ESCAPE THROUGH THE POINT OF ENTRY. SHOULD YOU LOOSE A LOT OF LIQUID THE INJECTION MUST BE REPEATED. WOUND CLIPS CAN HELP TO REDUCE THIS LEAKAGE.

10. PLACE MOUSE BACK IN CAGE TO RECOVER. A HEAT LAMP IS VERY BENEFICIAL TO HELP IN RECOVERY AS THE BODY TEMP OF THE MOUSE DECREASES WHILE UNDER THE INFLUENCE OF METAPHANE.

11. DO NOT RETURN THE MOUSE TO THE ANIMAL FACILITY UNTIL YOU ARE SURE IT IS UP AND MOVING ABOUT FREELY.

12. CHECK EACH MOUSE WITHIN 4-8 HOURS AFTER INJECTION TO BE SURE THERE ARE NO COMPLICATIONS. IF THE MOUSE IS HAVING DIFFICULTY REMOVE TO A SEPARATE CAGE AND USE A HEAT LAMP TO FACILITATE RECOVERY. BE SURE TO RECORD ANY UNUSUAL SYMPTOMS THAT MIGHT AFFECT THE INJECTION SUCH AS WOUNDS FROM OTHER MICE AROUND THE INJECTION SITE, ETC.

13. LENGTH OF TIME BEFORE SAW TUMOR: 2 WEEKS (A1N4-TH CELLS, B5 CLONE M/5FF)

A. MALLINCKRODT VETERINARY DIVISION, 421 HAWLEY STREET, MUNDELEIN, ILL (708) 949-3300

Section 4

SCORING GROWTH IN AGAR

SCORE	DESCRIPTION	EXPLANATION
NG	**No Growth**	Cells remained single as plated. No doubling or change of any kind seen.
LG	**Limited Growth**	Cells did manage to reproduce and form very small colonies of 5-20 cells each, after which no more growth was seen. This limited growth is considered as showing no growth in agar.
SG	**Small Growth**	Cells did grow to a scorabe colony size (50-60 cells)but did so very slowly. These clones do possess the ability to grow but seem to be lacking a particular requirement that would allow them to grow at their same rate as a monolayer.
G	**Growth**	Cells falling into this category comprise a very large range of growth from small to medium size colonies and form 1-2 colonies per dish to 10-20 per dish in a period of 4-8 weeks. Clearly, some cells were able to grow at the same rate as cells in monolayer.
G !	**good Growth**	Only a very few cells received this rating. The cells had to produce both many large colonies, and exhibit rapid growth in agar, forming colonies observable by eye within 1-3 weeks.

SELECTION OF $6TG^r$ / OUA^r DOUBLE MUTANTS

(Following procedure used by Dr. Regine Goth-Goldstein)

A mutant resistant to both 6-thioguanine (6-TG) and ouabain (OUA) was selected stepwise. First a $6TG^r$ mutant was selected after mutagen treatment. This was grown up to mass culture, treated again with mutagen and then $6TG^r$/OUA^r colonies were selected.

1. A $75cm^2$ flask of M10 cells in logarithmic growth was treated with 100ug/ml ENU (N-ethyl-N-nitrosourea[A] dissolved at 100 mg/ml in DMSO) in medium without serum for 90 minutes at 37 degrees C. Then the medium was removed and replaced with MEM medium + 10% calf serum and returned to the incubator for 3 days to recover.
2. After 3 days, the treated cells were trypsinized and plated at a concentration of 10^5 cells/100mm dish into 6TG medium (5.0 ug/ml 6TG) or at a concentration of 10^6 cells per dish in OUA medium (1uM)[B]
3. Surviving colonies should appear within 10 days. If no colonies are seen within 2 weeks repeat experiment. Surviving colonies are grown up in selection medium.

A. Pfaltz & Bauer Inc, cat no. N11970 (kept stored in a dessicator in the -20 degree freezer).

B. Note that this OUA concentration is much lower than that used in rodent cultures.

Section 6A

TECHNIQUE TO FUSE CELLS

(used by Dr. Regine Goth-Goldstein to fuse CHO and HeLa cells)

1. Early in the day, plate the 2 cell lines to be fused in a 100mm dish, about 10^6 cells each. Allow 4-8 hours to attach.
2. After cells have attached to the dish, remove medium
3. Rinse with 3cc PBS/dish
4. Treat with 1cc/dish of fusion medium:
   42% PEG
   10% DMSO
   make up in MEM
5. Treat a control set with only PBS
6. Leave fusion medium on for 1 minute
7. Immediately after treatment:
   a) Remove PEG solution
   b) Rinse 3X with PBS
   c) Rinse with 3cc medium
   d) Add 10cc medium (MEM 10% sera)
   e) Return to incubator
8. Allow 24 hours for the cells to recover from treatment
9. Remove MEM medium. Trypsinize cells and plate at 2-3 x 10^5 cells/100mm dish into selection media (listed below)
10. Check for colonies. You should see colonies within 4-8 days. If you do not see colonies after 2 weeks, the experiment was unsuccessful.
11. If colonies are seen, clone and re-plate into selection media. If cells continue to grow they are presumed to be fused. A colony forming ability test in the selection media should be done soon after selection. The fused cells tend to be larger so the setting on the coulter counter may need to be adjusted for a correct cell count.

Section 6B

PROCEDURE USED TO OBTAIN FUSED HMEC HYBRID

(Modified from RGG and R.H. Kennett (Methods in enzymology, vol. LVIII pg. 345)

1. Trypsinize cells to be fused 3 x 10^6 cells /each for treatment. Use 10^6 for control (no treatment)
2. Spin 10 min. at 1000 RPM. Remove supernate and retriterate in MEM-CS.
3. Spin for 10 min. at 1000 RPM, remove supernate.
4. Add 0.2cc fusion solution to tube and note the time added. Retriterate. Add 0.2cc PBS to control.
5. Spin 5 min. at 1000 RPM. Wait until solution is on cells for 8 min. before removing.
6. At 8 min. add 5cc MEM-CS. Retriterate.
7. Add MEM + 10% CS. Retriterate.
8. Spin 5 min. at 1000 RPM and remove supernate.
9. Resuspend in MEM + 10% CS and add to 100mm dishes filled with MEM + 10% serum. Plate about 2x10^5 cells per 100mm dish. I plated a series of dishes containing 2, 4, 6 x10^5 cells/dish.
10. Allow the cells to recover for 24 hours. After 24 hours check cells. If the cells look healthy enough, remove MEM and add selection media. (Selection media was added to our cells after 24 hours).
11. After 4 days in selection media, clumps of cells about 5-10 cells each could be seen. RGG felt these cells may not be true fused cells and were surviving due to membrane communication between non-fused cells. We trypsnized all cells and re-plated into selection media at a concentration of 1, 2, & 3x 10^5 cells per 100mm dish.

12. After 12 days in selection media many colonies could be seen. The larger, more healthy ones were marked. Most of these colonies were thin and grew in tight colonies that had opaque centers indicating a first level of transformation. There was one colony that had regular, square-shaped cells more typical of a normal HMEC cell.

13. 4 weeks after 2nd trypsinizing (step 9) several colonies were cloned into separate 60mm dishes containing selection media. In particular, most of the colonies came from one dish that had originally been plated with $2x10^5$ cells.
14. Of all the clones taken, only the one with the more normal phenotype survived and was confluent within 1 week after plating into a 60mm dish.

Section 6C

Media used to select a fused cell hybrid of a cross between control clone ($6TG^r/OUA^r$) x transformed clone ($6TG^s/OUA^s$)

Into a 500cc bottle of complete MEM media with 10% sera, add:

5cc 100x HAT concentrate[1]
0.5cc 1000x OUA (1mg/cc) for a final concentration of 1ug/cc
0.5cc 1000x Glycine (1mM) for a final concentration of 1uM

[1] GIBCO cat no. 31062-01 (light sensitive, store at 2-8 degrees C)

Section 6D

CLONES USED IN SUCCESSFUL CELL FUSION

I. NON-TRANSFORMED CLONE

Originally from Media Variant M10. Irradiated with 300 rads[1] at passage 47. Selected for $6TG^r$ after irradiation, two colonies were cloned, colony #1 and colony #2. The clone we used was colony #1. At passage 52, cells were treated with 100ug/cc ENU, and a double mutant $OUA^r/6TG^r$ clone was obtained. At passage 55 this mutant was fused with the transformed clone. The notation at the time of fusion was:

B5/19.F5-1/35(M10).C/12.X3/2.$6TG^r$1/3.ENU/1.OUA^r/3

[1] (250 Kvp x-rays) Expt 493 irradiated on 5/18/92

II. TRANSFORMED CLONE

Clone ID # 24 with the following notation at the time of fusion:

B5/19.F5-1/32(M4).Fe/8.OC/2.A11/5

Originally from Media Variant M4. Received Fe irradiation at passage 32. An agar colony (#11) was originally cloned from an opaque colony that had been plated into agar. It grew very fast in agar and produced many large colonies in just a few weeks.
This clone was fused to the control clone at passage 5 for a total passage no. of 47.

TABLE I

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	**CLONE SUMMARY LISTED BY EXPERIMENTS**												
2										FURTHER CLONING			
3	EXPT NO.	DATE	ION	EN'RGY Mev/u	DOSE (RADS)	I.D. CLONE IRRADIATED	2ND I.D.	CLONE SERIES PRODUCED	2ND I.D.	LOW SERA	OPQ COL	AGAR	NOTES
4	364	1/14/88	Fe	600	220	B5/19. *		B5/19.F5-1(M10)					MOST STABLE OF ALL CLONES
5								B5/19.F5-5(M10)					NOT A STRONG CLONE, SLOW GROWTH NOT USED IN EXPTS
6						* TOT AL ACTUAL PASSAGES = 50 CALC AS FOLLOWS:		B5/19.F5-1(M5) (@pass 25)				A2-A15	DERIVED FROM F5-1(M10) GREW IN MEM 5% SERA, LEAST STABLE OF ALL CLONES
7						STAMPFER CELLS FROZEN DOWN AT PASS 25. LINDA ADDED ABOUT 6 TO THAT, SO TOTAL =		B5/19.F5-3(M5)					DERIVED FROM F5-1(M10) GREW IN 5% SERA, NOT REG USED (ONLY USED IN EXPT 443)
8						FROZEN=25 LINDA PASG=6 NO. PAS AFTR RECV'D=19 TOTAL=50		B5/19.F5-1(M4)(THIN) (@ p39)			(OC)	N	DERIVED FROM F5-1(M5) (SEE ABOVE) GREW IN 4% SERA AND SELECTED FOR THEIR "THIN" LOOK
9	none	6/8/89	Fe	600	220	B5/19.F5-1/18(M10)		B5/19.F5-1/18(M10).Fe	FF5-1-4				
10						B5/19.F5-1/17(M4)		B5/19.F5-1/17(M4).Fe	FF5-1-3			A14 A15	
11	443	11/15/89	Ar	570	300	F5-3/7(M5)		F5-3/7(M5).Ar	Z				
12						F5-1/23(M4)		F5-1/23(M4).Ar	X,B		OC	A1 A3	
13						F5-1/17(M4).Fe/4	FF5-1-3	F5-1/17(M4).Fe/4.Ar	Y,A			A1,3,7 A1,A2	PREV IRRAD Fe 6/8/89 A1,3,7 PLATED INTO AGAR 7/10/91, A1,A2 PLATED INTO AGAR 8/13/91
14	444	11/28/89	Fe	600	220	F5-1/27(M5)		F5-1/27(M5).Fe	X,C		OC4 OC5	A1 A3 A1,A2, A3	A1,A3 NOT FROM AN OC AGAR COLONIES A1-A3 ORIGINATED FROM OPAQUE COLONY 5
15						F5-1/26(M4)		F5-1/26(M4).Fe	Z				
16						F5-1/17(M4).Fe/6	FF5-1-3	F5-1/17(M4).Fe/6.Fe	Y				PREV IRRAD Fe 5/8/89

	A	B	C	D	E	F	G	H	I	J	K	L	M
17	468-A	11/1/90	Nb	600	200	F5-1/25(M5).A13/4		F5-1/25(M5).A13/4.Nb					SEE ABOVE EXP 264-SPONTANEOUS AGAR CLONES AT P 25 WHEN TESTED AFTER THW'D
18	471-A	12/6/90	La	593	250	F5-1/40(M5)		F5-1/40(M5).La					
19	476	4/18/91	Fe	600	220	F5-1/27(M5).Fe/8	C	F5-1/27(M5).Fe/8.Fe	C		OC1 OC2	(Y)	2nd Fe 444
20						F5-1/17(M4).Fe/4.Ar/4	A,Y(443	F5-1/17(M4).Fe/4.Ar/4.Fe	A			A2	2nd Fe IRRAD 6/8/89, Ar 443,
21						F5-1/23(M4).Ar/4	B	F5-1/23(M4).Ar/4.Fe	B		OC	A1,A2 A3,A5	Ar 443,
22	479	8/8-13/ 1991	NONE	DENSITY	STUDY	VAR CLONES PLATED SAME DENSITY AS FOR TRS EXPT		CLONED ANY OPAQUE COLONY IF SEEN, AND PLATED INTO AGAR					
23	481-A	10/10/91	Fe	600	220	F5-1/34(M10)		F5-1/34(M10).Fe			(OC)		FIRST IN A SERIES OF 4-Fe IRRADIATIONS
24						F5-1/32(M4)		F5-1/32(M4).Fe			OC	A11 A12 A13	FIRST IN A SERIES OF 4-Fe IRRADIATIONS
25	484-A	12/5/91	Fe	600	265	F5-1/34(M10).Fe/4		F5-1/34(M10).Fe/4.Fe			(OC)	N	1ST Fe 481-A
26						F5-1/32(M4).Fe/4		F5-1/32(M4).Fe/4.Fe			(OC)	A1-A5	1ST Fe 481-A
27	485-A	2/19/92	Fe	600	220	F5-1/34(M10).Fe/4.Fe/3		F5-1/34(M10).Fe/4.Fe/3.Fe			(OC)	N	1ST Fe 481-A, 2ND Fe 484-A
28						F5-1/32(M4).Fe/4.Fe/3		F5-1/32(M4).Fe/4.Fe/3.Fe					1ST Fe 481-A, 2ND Fe 484-A
29	487	3/17/92	Ar	400	400	F5-1/42(M10)		F5-1/42(M10).Ar					AKA F5-1/35.C/6 (ACTUALLY A CONTROL FROM EXPT 481-A) CLONE M4 NOT IRRAD-NOT ENOUGH CELLS
30	489-A	4/2/92	Fe	600	220	F5-1/34(M10).Fe/4.Fe/3.Fe/2		F5-1/34(M10).Fe/4.Fe/3.Fe/2.Fe			(OC)	N	1ST Fe 481-A, 2ND Fe 484-A, 3RD 485-A
31						F5-1/32(M4).Fe/4.Fe/3.Fe/2		F5-1/32(M4).Fe/4.Fe/3.Fe/2.Fe					1ST Fe 481-A, 2ND Fe 484-A, 3RD 485-A (THESE CELLS MAY NOT BE FROZEN DOWN)
32	490-A	5/6/92	Ne	425	475	F5-1/45(M10) AKA F5-1/35(M10).C/10		F5-1/45(M10).Ne			(OC)	A	CLONE IRRAD WAS A CONTROL FROM ONE OF THE ABOVE Fe SERIES

	A	B	C	D	E	F	G	H	I	J	K	L	M
33						F5-1/43(M4) AKA F5-1/32.C/11		F5-1/43(M4).Ne					CLONE IRRADIATED WAS A CONTROL FROM ONE OF THE ABOVE Fe SERIES
34	496	11/24/92	Fe	600	220	F5-1/34(M10).Fe/4.Fe/10	10F2	same....Fe/10.Fe	F10F2				CELLS THW'D OUT 10/21 FOR THIS EXPT (1P-WHT-
35						F5-1/34(M10).Fe/4.Fe/3.Fe/6	10F3	same....Fe/6.Fe	F10F3				CELLS THW'D OUT 10/21 FOR THIS EXPT (1R-ORA
36						F5-1/34(M10).Fe/4.Fe/3.Fe/2.Fe/5	10F4	same....Fe/5.Fe	F10F4				CELLS THW'D OUT 10/21 FOR THIS EXPT (1S-YEL
37						F5-1/25(M5).A13/8	A13	same....A13/8.Fe	FA13				CELLS THW'D OUT 10/21 FOR THIS EXPT (6N-RED
38						F5-1/25(M5).A14/8	A14	same....A14/8.Fe	FA14				CELLS THW'D OUT 10/21 FOR THIS EXPT (6O-BLU
39						F5-1/25(M5).A15/7	A15	F5-1/25(M5).A15/7.Fe	FA15				CELLS THW'D OUT 10/6 FOR THIS EXPT (4W-WHT
40						F5-1/32(M4).Fe/6.OC1/6	4F	F5-1/32(M4).Fe/6.OC1/6.Fe	F4F				CELLS THW'D OUT 10/6 FOR THIS EXPT (1I-RED
41						F5-1/23(M4).Ar/4.Fe/1.OC1/5.A1/11	B	F5-1/23(M4).Ar/4.Fe/1.OC1/5.A1/11.Fe	FB				CELLS THW'D OUT 10/6 FOR THIS EXPT (1J-BLU
42						F5-1/32(M4).Fe/4.Fe/6.OC/2.A2/5	2	same....A2/5.Fe	F2				CELLS IN CULTURE (CLONED FROM AGAR 9/14) FROM EXPT 481-A, 484-A
43						same.....A3/5	3	same....A3.5.Fe	F3				CELLS IN CULTURE (CLONED FRM AGAR 9/8)FROM EXPTS 481-A, 484-A
44						same....A4/5	4	same....A4/5.Fe	F4				CELLS IN CULTURE (CLONED FROM AGAR 9/14)FROM EXPTS 481-A, 484-A
45						same....A5.5	5	same....A5/5.Fe	F5				CELLS IN CULTURE (CLONED FROM AGAR 9/14)FROM EXPTS 481-A, 484-A
46						F5-1/32(M4).Fe/8.OC/2.A11/5	11	same....A11/5.Fe	F11				CELLS IN CULTURE (CLONED FROM AGAR 9/14)FROM EXPT 481-A
47						same....A12/5	12	MOLD SEEN AFTER IRRAD					CELLS IN CULTURE (CLONED FROM AGAR 9/11)FROM EXPT 481-A
48						same....A13/5	13	same....A13/5.Fe	F13				CELLS IN CULTURE (CLONED FROM AGAR 9/14)FROM 481-A

	A	B	C	D	E	F	G	H	I	J	K	L	M
49						F5-1/35(M10).C/10 .Ne/3.OC/3.A/4	10N	F5-1/35(M10).C/10 .Ne/3.OC/3.A/4.Fe	F10N				CELLS IN CULTURE (CLONED FROM AGAR 10/28/92)FROM EXPT 490-A
50	497	12/23/92	Ar	570	250	F5-1/34(M10).Fe/4.Fe/11	10F2(#4	F5-1/34(M10).Fe/4.Fe/11.Ar	A10F2				
51						same .Fe/4.Fe/10.Fe/1	F10F2 (#25)	same.Fe/4.Fe/10.Fe/1.Ar	AF10F2				
52						F5-1/34(M10).Fe/4.Fe/3.Fe/7	10F3 (#4)	same.Fe/4.Fe/3.Fe/7.Ar	AF10F3				
53						same . Fe/4.Fe/3.Fe/6.Fe/1	F10F3 (#25)	same.Fe/4.Fe/3.Fe/6.Fe/1.Ar	AF10F3				
54						F5-1/34(M10).Fe/4.Fe/3.Fe/2.Fe/6	10F4 (#3)	same.Fe/4.Fe/3.Fe/2.Fe/6.Ar	AF10F4				
55						F5-1/25(M5).A13/9	A13 (#7)	same.A13/9.Ar	AA13				
56						same.A13/9.Fe/1	FA13 (#29)	same.A13/9.Fe/1.Ar	AF13				
57						F5-1/25(M5).A14/9	A14 (#10)	same.A14/9.Ar	AA14				
58						same.A14/8.Fe/1	FA14 (#29)	same.A14/8.Fe/1.Ar	AFA14				
59						F5-1/25(M5).A15/8	A15 (#10)	same.A15/8.Ar	AA15				
60						same.A15/7.A15/7.Fe/1	FA15 (#31)	same.A15/8.Fe/1.Ar	AFA15				
61						F5-1/32(M4).Fe/6.OC1/7	4F (#6)	same.Fe/6.OC1/7.Ar	A4F				
62						same.Fe/6.OC1/6.Fe/1	F4F (#26)	same.Fe/6.OC1/6.Fe/1.Ar	AF4F				
63						F5-1/23(M4).Ar/4.Fe/1.OC1/5.A1/11..Fe/	FB (#30)	same.Ar/4.Fe/1.OC1/5.A1/11.Fe/1.Ar	AFB				
64						F5-1/32(M4).Fe/4.Fe/6.OC/2.A2/6	2 (#8)	same.Fe/4.Fe/6.OC/2.A2/6.Ar	A2				
65						same.Fe/4.Fe/6.OC/2.A2/5.Fe/1	F2 (#28)	same.Fe/4.Fe/6.OC/2.A2/5.Fe/1.Ar	AF2				
66						same.same.A3/5.Fe/1	F3 (#31)	same.A3/5.Fe/1.Ar	AF3				
67						same.same.A4/6	4 (#9)	same.A4/6.Ar	A4				
68						same.same.A4/5.Fe/1	F4 (#26)	same.A4/5.Fe/1.Ar	AF4				
69						same.same.A5/6	5 (#9)	same.A5/6.Ar	A5				
70						same.same.A5/5.Fe/1	F5 (#28)	same.A5/5.Fe/1.Ar	AF5				

	A	B	C	D	E	F	G	H	I	J	K	L	M
71						F5-1/32(M4).Fe/8.OC/2.A11/6	11 (#11)	same.Fe/8.OC/2.A11/6.Ar	A11				
72						same.same.A11/5.Fe/1	F11 (#27)	same.same.A11/5.Fe/1.Ar	AF11				
73						same.same.A12/6	12 (#7)	same.same.A12/6.Ar	A12				
74						same.same.A13/6	13 (#8)	same.same.A13/6.Ar	AA13				
75						same.same.A13/5.Fe/1	F13 (#27)	same.same.A13/5.Fe/1.Ar	AF13				
76						F5-1/35(M10).C/10.Ne/3.OC/3.A/5	10N (#2)	same.Ne/3.OC/3.A/5.Ar	A10N				
77						same.Ne/3.OC/3.A/4.Fe/	F10N (#24)	same.Ne/3.OC/3.A/4.Fe/1.Ar	AF10N				
78					100	F5-1/35(M10).Fe/12	10F (#1)	F5-1/35(M10).Fe/12.Ar	A10F				
79					100	F5-1/45(M10).Ne/3.OC/3.A1/2	10N.A1 (#1)	F5-1/45(M10).Ne/3.OC/3.A1/2.Ar	A10N.A1				
80						F5-1/42(M10).Ar/4	10A (#2)	F5-1/42(M10).Ar/4.Ar	A10A				
81						F5-1/25(M5).A13/6.A/2	A13.A (#3)	MOLD CONTAMIN BEFORE CELLS FROZEN DOWN					
82						F5-1/23(M4).Ar/4.OC/4	4AOC (#5)	F5-1/23(M4).Ar/4.OC/4.Ar	A4AOC				
83						F5-1/23(M4).Ar/7	4A (#5	F5-1/23(M4).Ar/7.Ar	A4A				
84						F5-1/17(M4).Fe/4.Ar/7.A1/5	4FA (#6	F5-1/17(M4).Fe/4.Ar/7.A1/5.Ar	A4FA				

TABLE II

SCHEDULE OF TESTS AND RESULTS

CLONE ID NO.	DATE THAW'D	CELL/CLONE I.D. PASSAGE NO AT TEST (B5/19.)	DATE LOW SERA TEST PLATE/FIX		TEST NO	P.E. 1 %	P.E. 10%	%C 1%	DATE IN AGAR	TEST NO.	GROWTH RESULT	NO WKS	DATE FRZ	NO VLS	CAP I.D.	COL	NOTES/ LN2 LOC
COMPARISON - NUMBER OF TIMES IRRADIATED WITH IRON																	
8	9/10/93	F5-1/43(M4)	09/17	10/15	N5		1.97		09/17	N5	SG	5	10/28	?	4C/14	GRN	
"	"	" (TESTS IN BME)	"	"	"	0.03	2.52	0.978									
8	9/21/94	F5-1/32(M4)	09/26	10/11	N8	0	17.18	0.00	09/26	N8	G	6	10/14	2	M/4F	BLU	12/6
10	8/30/93	F5-1/17(M4).Fe/14	09/09	09/30	N3		5.16		09/09	N3	LG	6	09/09	1	FF/26	YEL	
"	"	" (TESTS IN BME)	"	"	"	0.61	30.02	3.86									
10	10/27/94	F5-1/17(M4).Fe/12	11/01	11/20	N12	0	19.12	0.00	11/01	N12	G	8	11/03	2	FF/24	ORA	12/6
15	9/21/94	F5-1/26(M4).Fe/5	09/29	10/24	N9	<.01	27.62	1.00	09/29	N9	mold		10/14	2	M/4FF	BLU	12/6
15	"	"	10/12	10/31	N11	<.01	40.86	<.01	10/12	N11	LG	5	already	fzn	down		10/14
24	9/10/93	F5-1/32(M4).Fe/10	09/17	10/15	N5		37.60		09/17	N5	LG	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	6.95	55.78	12.46									
24	10/27/94	F5-1/32(M4).Fe/9	11/08	12/02	N14	1.58	81.65	1.94	11/08	N14	G	9	11/10	2	4F	BLU	12/6
26	8/30/93	F5-1/32(M4).Fe/4.Fe/8	09/09	09/30	N3		18.65		09/09	N3	NG	6	09/09	1	4FF	ORA	
"	"	" (TESTS IN BME)	"	"	"	4.26	36.19	11.76									
26	10/27/94	F5-1/32(M4).Fe/4.Fe/7	11/03	11/23	N13	1.19	19.03	6.25	11/03	N13	G	7	11/08	2	4FF	WHT	
28	"	F5-1/32(M4).Fe/4.Fe/3.Fe/3	11/15	12/06	N16	8.83	39.46	22.38	11/15	N16	LG	8	11/18	2	4FFF	WHT	
16	9/21/94	F5-1/17(M4).Fe/6.Fe/6	09/26	10/11	N8	<.01	5.72	<.01	09/26	N8	SG	6	10/07	2	M/4FFF	YEL	12/6
31	NOT FROZN	F5-1/32(M4).Fe/4.Fe/3.Fe/2.Fe/															
6	9/17/93	F5-1/32(M5)	09/21	10/26	N6		3.15		09/21	N6	LG	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	0.03	14.67	0.168									
6	9/21/94	F5-1/29(M5)	09/26	10/11	N8	0	17.41	0.00	09/26	N8	G	6	10/07	2	M/5F	GRN	12/6
14	9/21/94	F5-1/27(M5).Fe/7	09/29	10/24	N9	3.64	47.76	7.62	09/29	N9	G	7	10/12	2	M/5FF	WHT	INJ
		" Fe/9	12/24	01/12	N22	19.2	57.77	33.15	02/24	N22	G!	10	already	fzn	down		N MOUSE
19	9/21/94	F5-1/27(M5).Fe/8.Fe/2	09/29	10/24	N9	<.01	23.28	1.50	09/29	N9	SG	6	10/12	2	M/5FFF	ORA	12/6
4	9/10/93	F5-1/44(M10)	09/16	10/11	N4		24.80		09/16	N4	SG	5	10/28	?	10C/13	WHT	
"	"	" (TESTS IN BME)	"	"	"	6.12	30.94	19.76									
4	9/21/94	F5-1/29(M10)	09/26	10/11	N8	<.01	10.57	<.01	09/26	N8	NG	6	10/07	2	M/10F	YEL	12/6
9	9/21/94	F5-1/18(M10).Fe/6	10/07	10/24	N10	<.01	15.05	<.01	10/07	N10	LG	6	10/07	2	M/10FF	ORA	12/6

9	"	" .Fe/7 (2nd freeze)											10/12	2	M/10FF	YEL	12/6
23	8/10/93	F5-1/34(M10).Fe/10 (TESTS IN BME)	08/17	09/07	N1	15.8	43.37	36.48	08/17	N1	LG	9	08/27	2	10Fe/11	GRN	IN
23	"	" Fe/12	09/16	10/11	N4		0.00										
"	"	" (TESTS IN BME)	"	"	"	6	50.88	11.79									
23	9/17/93	F5-1/34(M10).Fe/13	09/21	10/11	N6		33.10		09/21	N6	SG	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	8.3	43.30	19.17									
23	10/27/94	F5-1/35(M10).Fe/11	11/01	11/20	N12	3.14	43.69	7.19	11/01	N12	NG	8	11/03	2	10Fe/11	YEL	S
25	8/30/95	F5-1/34(M10).Fe/4.Fe/11	09/09	09/30	N3		37.89						09/09	1	10FF	BLU	
"	"	" (TESTS IN BME)	"	"	"	10.5	41.19	25.42	09/09	N3	NG	6					
25	9/21/94	F5-1/34(M10).Fe/4.Fe/6	09/26	10/11	N8	1.4	39.98	3.42	09/26	N8	G	6	10/07	2	M/10FFF	RED	12/6
27	9/17/93	F5-1/34(M10).Fe/4.Fe/3.Fe/4	09/21	10/15	N6		22.00		09/21	N6	NG	5	10/28	2	10FFF	RED	
"	"	" (TESTS IN BME)	"	"	"	4.39	30.55	14.37									
27	10/27/94	F5-1/35(M10).Fe/4.Fe/3.Fe/3	11/01	11/20	N12	5.28	40.58	13.01	11/01	N12	NG	8	11/03	2	10FFF	GRN	S
34	"	F5-1/35(M10).Fe/4.Fe/10.Fe/1	11/03	11/23	N13	2.07	25.12	8.24	11/03	N13	G	7	11/08	2	10FFF	ORA	INJ
34	"	"	12/28	01/12	N24	8.45	56.56	14.94	12/28	N24	G	9	already	fzn	down		N MOUSE
30	9/17/93	F5-1/34(M10).Fe/4.Fe/3.Fe/2.Fe/5	09/21	10/15	N6		29.53		09/21	N6	LG	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	6.23	36.96	16.86									
30	"	F5-1/35(M10).Fe/4.Fe/3.Fe/2.Fe/2	11/03	11/23	N13	15	57.59	26.05	11/03	N13	G	7	11/08	2	10FFFF	RED	INJ
30	"	" Fe/3	12/07	12/28	N20	13.3	77.75	17.75	12/07	N20	G	9	already	fzn	down		INTO
30	"	" Fe/4	12/15	01/04	N21	12.9	59.65	21.60	12/15	N21	G	12	already	fzn	down		NUDE
35	"	" Fe/2.Fe/4	12/24	01/12	N22	11.4	54.29	20.99	12/24	N22	G	12	already	fzn	down		MOUSE
35	"	F5-1/35(M10).Fe/4.Fe/3.Fe/6.Fe/1	11/01	11/20	N12	1.59	24.79	6.41	11/01	N12	LG	8	11/03	2	FF3	BLU	S
36	"	F5-1/35(M10).Fe/4.Fe/3.Fe/2.Fe/5.Fe/	11/08	12/02	N14	1.68	56.50	2.98	11/08	N14	SG	9	11/16	4	F10F4	BLU	INJ
36	"	" Fe/2	11/21	12/16	N18	9.29	62.90	14.77	11/21	N18	G	9	already	fzn	down		NUDE
36	"	" Fe/3	12/06	12/24	N19	9.34	50.97	18.34	12/06	N19	G	10	already	fzn	down		MOUSE
COMPARISON - EFFECT OF DIFFERENT IONS WITH SINGLE IRRADIATION																	
10	10/27/94	F5-1/17(M4).Fe/12	11/01	ABOVE	N12			0.00	11/01	N12	G	8					
15	9/21/94	F5-1/26(M4).Fe/5	09/29	ABOVE	N9			1.00	10/12	N11	LG	5					
24	10/27/94	F5-1/32(M4).Fe/9	11/08	ABOVE	N14			1.94	11/08	N14	G	9					
12	8/24/93	F5-1/32(M4).Ar/7	08/26	09/16	N2		4.90		08/26	N2	NG	8	08/26	1	B	GRN	3N
"	"	" (TESTS IN BME)	"	"	"	0.17	6.90	2.48									
12	9/10/93	F5-1/23(M4).Ar/4	09/16	10/11	N4		6.35		09/16	N4	G	5	09/16	1	?	?	
"	"	" (TESTS IN BME)	"	"	"	0.14	10.44	1.43									
"	"	" Ar/6 (2ND FREEZE)											10/28	?	443-F5-1	YEL	
12	11/10/94	F5-1/23(M4).Ar/6	11/20	12/06	N17	0.95	11.73	8.06	11/20	N17	NG	9					1/5

33	11/10/94	F5-1/43(M4).Ne/3	11/16	12/06	N16	0.95	3.90	24.38	11/16	N16	G	8	11/18	2	4Ne/5	RED	
14	9/21/94	F5-1/23(M5).Fe/6	09/29	ABOVE	N9			7.62			G	7					
11	9/10/95	F5-3/7(M5).Ar/7	09/16	10/11	N4		0.00		09/16	N4	SG	5	09/16	1	?	?	
"	"	" (TESTS IN BME)	"	"	"	0.1	0.48	20.78									
11	11/10/94	F5-3/11(M5).Ar/6	11/16	12/06	N16	0	0.90	0.00	11/16	N16	NG	8	11/18	2	5Ar	YEL	
18	9/10/93	F5-1/40(M5).La/4	09/17	10/15	N5		2.00		09/17	N5	LG	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	0.54	2.87	18.65									
18	11/7/94	F5-1/40(M5).La/3	11/16	12/06	N16	0.3	2.09	14.52	11/16	N16	SG	8	11/18	2	5La	ORA	
9	9/21/94	F5-1/18(M10).Fe/6	10/07	ABOVE	N10			<.01			LG	6	already	fzn	down		
23	10/27/94	F5-1/35(M10).Fe/11	11/01	ABOVE	N12			7.19			NG	8	already	fzn	down		
29	NOT FROZN	F5-1/32(M10).Ar															
32	8/10/93	F5-1/45(M10).Ne/5 (TESTS IN BME)	08/17	09/07	N1	14.1	42.38	33.20	08/17	N1	LG	9	08/27	2	10Ne/6	BLU	1U
32	"	" Ne/7 (TESTS IN BME)	09/09	09/30	N3	2.36	29.65	7.96									
32	11/7/94	F5-1/43(M10).Ne/3	11/10	12/06	N15	3.39	33.09	10.24	11/10	N15	NG	9	11/15	2	10Ne/5	YEL	
COMPARISON - PROGRESSION OF TRANSFORMATION AFTER SINGLE, DOUBLE IRRADIATION																	
24	10/27/94	F5-1/32(M4).Fe/9	11/8	ABOVE	N14			1.94			G	9					
24	8/10/93	F5-1/32(M4).Fe/6.0C1/7	8/17	9/7	N1	3.71	23.7	15.69	8/17	N1	LG	9	8/27	1	4Fe/12	WHT	1S
24	12/5/94	F5-1/32(M4).Fe/8.OC1/2	12/24	1/12	N22	1.23	12.3	9.99	12/24	N22	SG	10	12/28	2	M4 F9	GRN	
24	12/5/94	F5-1/32(M4).Fe/8.OC1/2.A11/4	12/15	1/4	N21	<0.1	100.0	<0.1	12/15	N21	G!!!	13	12/24	2	4F11	GRN	N MOUSE
24	12/5/94	F5-1/32(M4).Fe/8.OC1/2.A12/4	12/15	1/4	N21	0	83.2	0	12/15	N21	G	13	12/24	2	4F12	BLU	
24	12/5/94	F5-1/32(M4).Fe/8.OC1/2,A13/4	12/15	1/4	N21	0	76.6	0	12/15	N21	G	13	12/24	2	4F13	YEL	
26	10/27/94	F5-1/32(M4).Fe/4.Fe/7	11/03	ABOVE	N13			6.25	11/03	N13	G	7	11/08				
26	11/15/94	F5-1/32(M4).Fe/4.Fe/6.OC/2.A3/4	12/06	12/24	N19	0.8	4.91	32.35	12/06	N19	NG	10	12/08	2	46/6	BWN	
12	11/10/94	F5-1/23(M4).Ar/6	11/20	ABOVE	N17				11/20	N17	NG	9	11/22	2	4Ar	ORA	
12	11/15/94	F5-1/23(M4).Ar/4.OC/3	12/06	12/24	N19	<.1	1.64	<.1	12/06	N19	LG	10	12/08	2	OCB	GRN	1/5
12	11/15/94	F5-1/23(M4).Ar/7.A1/5	11/20	12/16	N17	0	1.54	9.48	11/20	N17	LG	9	11/22	2	4ArA1	WHT	1/5
6	12/19/94	F5-1/23(M5).A13/5	12/28	01/12	N24	0	3.72	0.00	12/28	N24	G	9	01/05	2	5A13	ORA	
14	8/24/93	F5-1/27(M5).Fe/10	08/26	09/16	N2		11.50		08/26	N2	NG	8	08/26	1	C	YEL	3P
"	"	" (TESTS IN BME)	"	"	"	3	15.81	19.54									
14	9/21/94	F5-1/27(M5).Fe/6	09/29	ABOVE	N9			7.62	09/29		G	7	10/12				
14	8/16/93	F5-1/27(M5).Fe/10.A3/8	08/26	09/16	N2		4.95										
"	"	" (TESTS IN BME)	"	"	"	0	15.81	0.00	08/26	N2	NG	8	08/26	1	C3	WHT	1E
"	"	" A3/10	09/17	10/15	N5		0.20		09/17	N5	LG	5	already	fzn			
"	"	" (TESTS IN BME)	"	"	"	0	0.47	0.00									
14	8/10/93	F5-1/27(M5).Fe/10.A1/8	08/17	09/07	N1	0	0.00	0.00	08/17	N1	NG	9	08/27	1	C-1	YEL	1K

14	"	" A1/11	09/09	09/30	N3		0.78		09/09	N3	SG	6	already	fzn			
"	"	" (TESTS IN BME)	"	"	"	0	0.49	0.00									
14	11/15/94	F5-1/27(M5).Fe/10.A1/7	11/21	12/16	N18	0	0.16	0.00	11/21	N18	G	9	11/23	2	5FA1	BLU	1/5
14	11/15/94	F5-1/27(M5).Fe/6.OC5/3	11/21	12/16	N18	11.7	59.31	19.65	11/21	N18	NG	9	11/23	2	5FOC	YEL	
14	11/15/94	F5-1/27(M5).Fe/6.OC5/2.A1/6	12/06	12/24	N19	1.4	8.49	16.49	12/06	N19	G	10	12/08	2	C0C5-1	GRY	
19	9/21/94	F5-1/27(M5).Fe/8.Fe/2	09/29	ABOVE	N9			1.50	09/29	N9	SG	6					
19	11/15/94	F5-1/27(M5).Fe/8.Fe/1.OC2/3	11/20	12/16	N17	8.65	20.43	42.34	11/20	N17	LG	9	11/22	2	5FFOC	GREY	
32	11/7/94	F5-1/45(M10).Ne/3	11/10	ABOVE	N15			10.24	11/10	N15	NG	9					
32	11/15/94	F5-1/45(M10).Ne/3.OC/3.A/3	11/21	12/16	N18	0.09	24.21	0.40	11/21	N18	NG	9	11/23	2	10NeOCA	ORA	1/5
COMPARISON-ADDITIONAL IRRADIATION OF A CLONED AGAR OR OPAQUE COLONY																	
40	12/19/94	F5-1/32(M4).Fe/6.OC1/6.Fe/2	12/28	1/12	N23	<.1	15.6	0.17	12/28	N23	SG	13	1/5	2	F4F	GRN	
41	12/19/94	F5-1/23(M4).Ar/4.Fe/1.OC1/5.A1/11.Fe/2	12/28	1/12	N23	<.1	57.5	<.1	12/28	N23	G!	13	1/5	2	FB	BLU	
37	12/19/94	F5-1/25(M5).A13/8.Fe/2	12/28	1/12	N23	<.1	9.8	<.1	12/28	N23	SG	13	1/5	2	FA13	GRN	
17	9/10/93	F5-1/25(M5).A13/4.Nb/5	9/17	10/15	N5		4.3		9/17	N5	G	5	NOT FZN				
"	"	" (TESTS IN BME)	"	"	"	0	0.2	0									
17	12/19/94	F5-1/25(M5).A13/4.Nb/2	12/31	1/28	N25	0	1.6	0	12/31	N25	LG	13	1/5	2	513 Nb	YEL	
49	12/19/94	F5-1/45(M10).Ne/3.OC/3.A/4.Fe/	12/28	1/12	N24	1.76	23.5	2.96	12/28	N24	G	2	1/5	2	FION	RED	
MISC CLONES																	
21	8/10/93	F5-1/23(M4).Ar/4.Fe/1.OC-1/5.A5/8	8/17	9/7	N1	1.34	8.0	16.85	8/17	N1	NG	9	8/27	1	OC1-5	RED	1F
21	8/16/93	F5-1/23(M4).Ar/4.Fe/10.A3/9	8/26	9/16	N2		24.8		8/26	N2	LG	8	8/26	1	B	BLU	1L
"	"	" (TESTS IN BME)	"	"	"	0.05	6.9	2.48									
21	8/24	F5-1/23(M4).Ar/4.Fe/1.OC1/5.A1/9	9/9	9/30	N3		1.2		9/9	N3	SG	6	9/9	1	OC1-1/9	GRN	
"	"	" (TESTS IN BME)	"	"	"	0	1.9	0									
13	9/10	F5-1/17(M4),Fe/4.Ar/7	9/16	10/11	N4		0.4		9/16	N4	SG	5	9/16	1	A?	?9	
"	"	" (TESTS IN BME)	"	"	"	0	3.2	0									
	NOTE:	N7 IS NOT LISTED BECAUSE															
		NO B5F5 CELLS USED															
		(ONLY TESTED HTB126 CELLS)															

TABLE III

SUMMARY OF TEST RESULTS

PART A - EFFECT OF MULTIPLE IRRADIATION

CLONE NO. I.D.	PASSAGE NO. OF MEDIA VARIANT WHEN FIRST IRRAD	ORIGINAL MEDIA VARIANT	NO TIMES IRRAD WITH FE	%C PE IN 1% SERA	GROWTH IN AGAR		
8	32	M4	0	0	G		
24	32	M4	1	1.94	G		
26	32	M4	2	6.25	G		
28	32	M4	3	22.38	LG		
6	29	M5	0	0	G		
14	27	M5	1	7.62	G		
14	"	"	"	33.15	G!		
19	27	M5	2	1.5	SG		
4	35	M10	0	<.01	NG		
23	34	M10	1	7.19	NG		
25	35	M10	2	3.42	G		
27	35	M10	3	13.01	NG		
34	35	M10	3	8.24	G		
34	"	"	"	14.94	G		
30	35	M10	4	26.05	G		
30	"	"	"	17.75	G		
30	"	"	"	21.6	G		
35	"	"	"	20.99	G		
35	35	M10	4	6.41	LG		
36	35	M10	5	2.98	SG		
36	"	"	"	14.77	G		
36	"	"	"	18.34	G		

PART B - EFFECTIVENESS OF DIFFERENT IONS

CLONE NO. I.D.	PASSAGE NO. OF MEDIA VARIANT WHEN FIRST IRRAD	TOTAL NO. PASSAGES	MEDIA VARIANT	ION	%C PE IN 1% SERA	GROWTH IN AGAR	
24	32	41	M4	Fe	1.94	G	
12	23	29	M4	Ar	8.06	NG	
33	43	46	M4	Ne	24.38	G	
14	23	33	M5	Fe	7.62	G	
18	40	43	M5	La	14.52	SG	
23	18	46	M10	Fe	7.19	NG	
32	43	46	M10	Ne	10.24	NG	

TABLE III (CONTINUED)

PART C - EFFECT OF PASSAGE NO.

CLONE NO. I.D.	PASSAGE NO. OF MEDIA VARIANT WHEN FIRST IRRAD	TOTAL NO. PASSAGES	MEDIA VARIANT	ION	NO TIMES IRRAD	%C PE IN 1%	GROWTH IN AGAR
10	17	29	M4	Fe	1	0	G
15	26	31	M4	Fe	1	1	LG
15	"	31	"	"	"	<.01	G
24	32	41	M4	Fe	1	1.94	G
16	17	29	M4	Fe	2	<.01	SG
26	32	43	M4	Fe	2	6.25	G
9	18	24	M10	Fe	1	<.01	LG
23	35	46	M10	Fe	1	7.19	NG

PART D - CLONES FROM TRANSFORMED STAGES

NUMBER ID	MEDIA VARIANT	DESCRIPTION	NO TIMES IRRAD	ION	%C IN 1%	GROWTH IN AGAR	STAGE CLONED
26	M4	Fe Fe	2	Fe	6.25	G	
26	M4	Fe Fe OC A3	2	Fe	32.35	NG	AGAR COLONY CLONED FROM AN OPAQUE COLONY
12	M4	Ar	1	Ar	8.06	NG	
12	M4	Ar OC	1	Ar	<0.1	LG	OPAQUE COLONY
12	M4	Ar OC A1	1	Ar	9.48	LG	AGAR COLONY CLONED FROM AN OPAQUE COLONY
14	M5	Fe	1	Fe	33.15	G!	INJ NUDE MICE CAUSED SM TUMORS
14	M5	Fe A1	1	Fe	0	G	AGAR COLONY
14	M5	Fe OC	1	Fe	19.65	NG	OPAQUE COLONY
14	M5	Fe OC A1	1	Fe	16.49	G	AGAR COLONY CLONED FROM AN OPAQUE COLONY
19	M5	Fe Fe	2	Fe	1.5	SG	
19	M5	Fe Fe OC	2	Fe	42.34	LG	OPAQUE COLONY
32	M10	Ne	1	Ne	10.24	NG	
32	M10	Ne OC	1	Ne	0	NG	
24	M10	Fe	1	Fe	1.94	G	
24	M10	Fe OC	1	Fe	9.99	SG	OPAQUE COLONY
24	M10	Fe OC A	1	Fe	<.01	G!!! (BEST GROWTH IN AGAR)	AGAR COLONY CLONED FROM AN OPAQUE COLONY

TABLE III (CONTINUED)

PART E - EFFECT OF ADDITIONAL IRRADIATION OF CLONED OPAQUE AND AGAR COLONIES

CLONE NO. I.D.	MEDIA VARIANT	DESCRIPTION	%C PE IN 1%	GROWTH IN AGAR	NOTES		
24	M4	Fe	1.94	G	Single Fe	irrad	
24	M4	Fe OC1	9.99	SG	Opaque	Colony	
40	M4	Fe OC Fe	0.17	SG	Opaque	Colony Fe	irradiated
6	M5	A13	0	G	Agar	Colony 13	
37	M5	A13 Fe	0	SG	Agar	Colony Fe	irradiated
17	M5	A13 Nb	0	LG	Agar	Colony Nb	irradiated
32	M10	Ne	10.24	NG	Single Ne	irrad	
32	M10	Ne OC A	0.4	NG	Agar Col	from Opaq	Colony
49	M10	Ne OC A Fe	2.96	G	Agar	Colony Fe	irradiated

TABLE IV

SUMMARY OF RESULTS - TUMOR FORMATION IN NUDE MICE

CLONE I.D.	NO. MICE INJECTED	TOTAL NO. INJECTION SITES	TOTAL NO. CELLS INJECTED	NUMBER OF TIUMORS OBSERVED	NO. MICE WITH TUMORS	% MICE WITH TUMORS	% TUMOR FORMATION PER SITE
4F11	6	15	1.55×10^8	NONE	NONE	0	0
F10F4	6	15	1.5×10^8	NONE	NONE	0	0
10FFF	6	14	1.45×10^8	NONE	NONE	0	0
10FFFF	6	12	1.2×10^8	NONE	NONE	0	0
M/5 FF	**7**	**19**	**2.03×10^8**	**8**	**3**	**43%**	**42%**
A1N4-TH	6	14	1.45×10^8	14	6	100%	100%
A1N4-TH TUMOR CELLS	1	4	5.5×10^7	4	1	100%	100%