FAPESP 60 ANOS 1962 - 2022

GENOME 20+2

RICARDO RENZO BRENTANI
1937 – 2011

Messenger Activity of Purified RNA from Rat Liver Nuclei

Service to R. M. Featherstone, chairman, Department of Pharmacology, University of California, San Francisco.

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MESSENGER RNA, which is characterized by a DNA-like composition and has a high rate of turnover, has already been described in rat liver nucleoli[1,2] and calf thymus nucleoli[3].

All RNA species are synthesized on a DNA template and are thus complementary to their respective cistrons[4–6]. RNAs with a high rate of turnover have been found which exhibit no messenger activity[7], and some animal messengers have proved to be quite stable[8]. Because of these findings, some well defined criteria are required in order that a molecule can be unequivocally referred to as "messenger RNA".

One such criterion is the ability of messenger RNA to stimulate the incorporation of amino-acids by messenger depleted (pre-incubated[9]) ribosomes in reaction which is dependent on energy and sensitive to puromycin. This property is not shared by transfer RNA[10,11] or structural ribosomal RNA[12,13] unless the latter is denatured by boiling and neomycin added to the incubation mixture[14,15].

Messenger RNA has been found both in intact nuclei[16–18] and in isolated ribonucleoprotein fractions from rat liver nucleoli[19]. The present communication is concerned with some properties of an RNA purified from these fractions, and a comparison made with an RNA obtained from morphologically identifiable nucleoli.

Nuclei were prepared from Wistar rats which had been fasted for 24 h (both sexes, weighing 180–200 g) and the purity of the preparation was checked by phase microscopy and electron micrography[20].

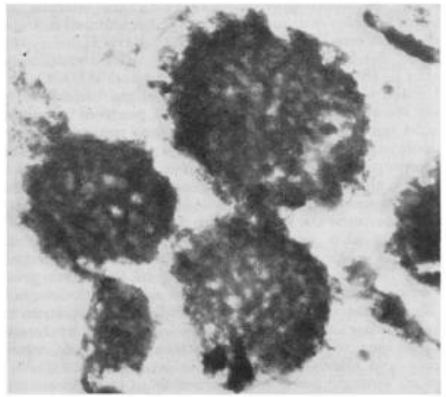

Fig. 1. Electron micrography of isolated nucleoli. Purified by sonic oscillation. (×6,000.)

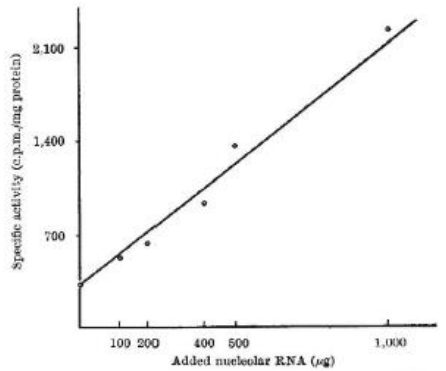

Fig. 2. Effect of nucleolar RNA (nucleoli prepared by sonic oscillation) on incorporation of ^{14}C-leucine by pre-incubated ribosomes. (The same experimental conditions as described in Table 1.)

Nucleoli were prepared either by sonication[20] or by salt fractionation of the nuclear fraction[21]. The first procedure yields two sub-nuclear components—the nucleolus and the extra-nucleolar fraction—whereas by the second procedure we obtain three components—the nucleolus, the chromatin and the nucleoplasm.

RNA was purified from these fractions according to the procedure of Harris[22], modified by the addition of 0·2 mg/ml. bentonite, and added in varying concentrations to pre-incubated ribosomes. These ribosomes were prepared by Korner's procedure "*b*"[23] and pre-incubated to promote messenger depletion[24].

It can be seen that nucleolar RNA is capable of stimulating the incorporation of amino-acids, and that the reaction is inhibited by puromycin to the same extent as normal incorporation. The lower stimulating activity of the RNA from nucleoli purified by sonic oscillation (Fig. 2) can be attributed to degradation and solubilization of messenger RNA. Similar events have been described for ribosomes and microsomes[25–29].

If it were possible to extrapolate to animal tissues recent data on *Drosophila*[30], which show complete saturation of nucleolus-associated chromatin with cistrons for 28*S* structural ribosomal RNA[30], it could be assumed that the nucleolar messenger RNA is synthesized in the chromatin region, and that later it migrates to the nucleolus where it

Table 1. EFFECT OF RNA FROM NUCLEAR SUBFRACTIONS ON INCORPORATION OF ^{14}C-LEUCINE BY PRE-INCUBATED RIBOSOMES

Additions	Specific activity (c.p.m./mg protein)
Normal ribosomes	415
+ 0·2 μmoles puromycin	129
− ATP PEP pyruvate kinase	68
− Mg^{++}	128
− GTP	104
− pH 5 enzyme	101
Pre-incubated ribosomes	227
+ 0·2 mg chromatin RNA	389
+ 0·2 mg nucleoplasmic RNA	954
+ 0·2 mg nucleolar RNA	2,199
+ 0·2 mg nucleolar RNA + 0·2 μmoles puromycin	772
+ 0·3 mg polyuridylic acid*	470

The reaction mixture contained, in a final volume of 1·5 ml., the following (unless otherwise specified): 0·8 mg pH 5 enzyme[25]; 10 μmoles magnesium chloride; 87·5 μmoles sucrose; 20 μmoles 0·05 molar potassium phosphate buffer pH 7·4; 2·5 μmoles ATP; 0·5 μmoles GTP; 20 μmoles phosphoenol pyruvate; 0·2 mg pyruvate kinase; 100 μmoles reduced glutathione; 0·1 μmole ^{14}C-leucine (~ 175·000 c.p.m.) and 2 mg ribosomal protein. Incubation was at 37° C for 120 min and the reaction stopped with 1·7 ml. 12 per cent ice-cold trichloracetic acid containing 0·2 per cent unlabelled leucine (phenylalanine in the experiment with poly U). Proteins were washed[24] and counted at infinite thinness in a thin-window, low-background gas-flow counter. Each value given is the mean of duplicates. A standard containing 0·01 μc. gave 2,000 c.p.m.

* ^{14}C-Leucine was replaced by 0·1 μmoles ^{14}C-phenylalanine (175,000 c.p.m.).

accumulates. Such a migration has already been suggested[31,32].

We do not know whether this RNA codes for acidic protein synthesis, which presumably takes place in the nucleolus[33–36], or for cytoplasmic proteins—the nucleolar stage is necessary to its association with newly synthesized ribosomes—or whether the 45*S* precursor to structural ribosomal RNA[4,37–40] is single stranded, and can thus stimulate the incorporation of amino-acids without being a true messenger.

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In this communication some evidence is presented about the nature of the ribosomal aggregates which are believed to be identical to the Nissl granules.

White Wistar rats aged from 10 days to 5 months received 10 μc. of ^{14}C-leucine (U) intraperitoneally from 5 to 30 min before decapitation in the *in vivo* protein synthesizing experiments. The brains were quickly homogenized at 0° C in a medium containing 0·05 molar *tris* buffer, *p*H 7·4, 5 × 10^{-3} molar magnesium chloride, 0·025 molar potassium chloride, 0·25 molar sucrose. The 15,000*g* postmitochondrial supernatant was fractionated in a 10–30 per cent linear sucrose density gradient containing a 50 per cent sucrose step prepared in the foregoing medium at 63,000*g* for 2 h. In the *in vitro* protein synthesizing system, heavy and light microsomes were prepared by centrifuging the ribosomes for 1 h at 25,000*g* and 105,000*g*, respectively, and incubating for 10 min in

Role of Nucleolar Ribonucleic Acid in Incorporation of Ribosomal Amino-acid

It is known that incorporation of amino-acids into protein by ribosomes is dependent on RNA, and thus sensitive to RNase[1]. It is believed that this incorporation requires a 'messenger RNA', which may have a nucleolar origin[2].

To test this hypothesis we measured the effect of nucleolar RNA, prepared according to Allfrey *et al.*[3], on ribosomes treated with RNase (they lost 75 per cent of their RNA this way).

Table 1 gives the results of 12 experiments, which show that incorporation of either ^{14}C-glycine or ^{14}C-valine is enhanced by the addition of nucleolar fraction. Pretreatment of this fraction with RNase destroyed its activity.

Table 1. Effect of Nucleolar RNA on Amino-acid Incorporation

No.	Ribosome	Nucleolar fraction	Ribosome + nucleolar fraction	Ribosomes + RNase-treated nucleolar fraction
1	72	3	844	
2	21	100	320	
3	158	157	398	
4	20	41	474	
5	190	229	1,308	
6	223	246	670	
7	480	507	2,322	
8	383	448	935	383
9	269	333	821	369
10	321	234	652	
11	280	756	512	242
12	26	55	121	102

To 0·5 ml. of an incubating mixture containing 2 μmol. of ATP, 0·25 μmol. of GTP, 0·04 μmol. (90,000 c.p.m.) of ^{14}C-glycine or ^{14}C-valine (exps. 10, 11, 12), 10 μmol. of phosphoenolpyruvate, 12 μmol. of *tris* buffer *p*H 7·5 and 50 μg of crystalline pyruvate kinase, we added 6·5 mg of ribosomal protein (ref. 1) (in 0·5 ml. of *tris* buffer *p*H 7·5, 0·045 M), 1·5 mg of *p*H 5 enzyme (in 0·25 ml. of sucrose 0·44 M) and 0·4 mg of nucleolar RNA fraction (ref. 3) (in 0·5 ml. of 0·44 sucrose). The mixture was incubated for 2 h at 30° C and the reaction stopped by adding 1 ml. of cold 10 per cent TCA. Precipitated proteins were washed by the procedure of Siekevitz (ref. 8), modified by washing for 1 h at room temperature in N sodium hydroxide.

These results agree with Sibatani, de Kloet, Allfrey and Mirsky's hypothesis[2]. As a matter of fact Perry[4] demonstrated that some nucleolar RNA migrates to ribosomes. Recent papers[5–7] have shown that synthetic polyribonucleotides can stimulate amino-acid incorporation into pre-incubated microsomes, and it is reasonable to assume that the latter are depleted of active RNA.

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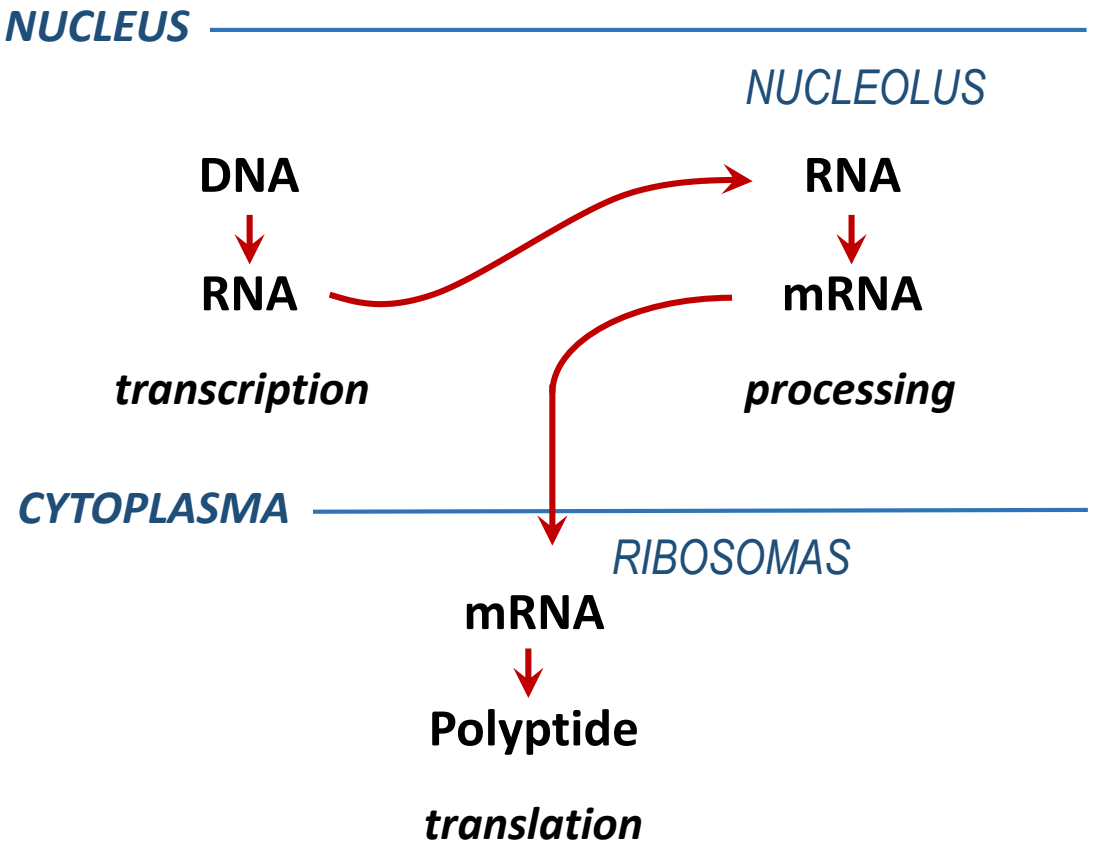
disoriented by concentrated urea solution[3], it seems that the molecular framework is primarily held together by hydrogen bonds; disulphide linkages are absent.

Only a few investigators have examined the properties of prekeratin *in vitro*. Rudall found that the buffered aqueous solvents commonly used for the extraction of proteins are unsatisfactory for the extraction of prekeratin. He noted, however, that copious amounts of protein can be removed from the epidermis with concentrated urea solution. In view of this, Rudall[4] selected 6 M urea solution for the extraction of prekeratin (epidermin) from the epidermis of cow's nose, and Rogers[5] used 8 M urea solution for the isolation of proteins from the wool root. Analyses of the extracts thus obtained showed that they contain globular-type proteins in addition to the precursor of fibrous keratin. Complete separation of these globular proteins from prekeratin has proved very difficult[6,7]. The partially purified prekeratin preparations were commonly found to have a relatively low sulphur content; furthermore, the bulk of the sulphur present was in the —SH form. The average molecular weight of the preparations was found to be in the range of 60,000–100,000, thus indicating that urea splits the macromolecules of prekeratin into relatively small units. So far as the shape and molecular structure of the isolated prekeratin are concerned, it appears significant that dry films of the preparations prepared from the urea extracts were anisotropic in polarized light and showed an α pattern in X-ray diffraction investigations[4,6].

The purpose of this communication is to report a new isolation technique by which pure prekeratin can be obtained from the epidermis of the cow's nose. This prekeratin has a high molecular weight and can be readily spun into fibres. For the isolation 0·1 M citric acid–sodium citrate buffer (CASC) of *p*H 2·6 and ionic strength of 0·6 was used.

After thorough washing of the cow's noses in running tap water, the epidermis is removed in sheets 0·5 mm thick with a keratotome (obtained from the Storz Instrument Co., St. Louis, 10, Missouri). The epidermal sheets are collected in a beaker, and, after addition of a few millilitres of CASC buffer, are cut into small pieces with scissors. The minced epidermis is afterwards suspended in CASC buffer in the ratio of 1 : 5 and homogenized in the 'VIRTIS' apparatus for 4 min. The homogenate is then filtered through gauze and centrifuged for 10 min at 3,500 r.p.m. The resulting supernatant is centrifuged for 30 min at 20,000 r.p.m. The supernatant of this centrifugation is then diluted 1 : 1 with CASC buffer and the *p*H adjusted to 7 by the addition of 1 N sodium hydroxide drop by drop and constant stirring with a magnetic bar. The first precipitates of prekeratin appear at about *p*H 3·6. The large, sticky fibrous precipitates formed at *p*H 7 have a tendency to fuse and form an insoluble clot. It is advisable, therefore, to decant the supernatant fluid as soon as the

Role of Nucleolar Ribonucleic Acid in Incorporation of Ribosomal Amino-acid



Science

Presence of Laminin Receptors in *Staphylococcus aureus*

Abstract. *A characteristic feature of infection by* Staphylococcus aureus *is bloodstream invasion and widespread metastatic abscess formation. The ability to extravasate, which entails crossing the vascular basement membrane, appears to be critical for the organism's pathogenicity. Extravasation by normal and neoplastic mammalian cells has been correlated with the presence of specific cell surface receptors for the basement membrane glycoprotein laminin. Similar laminin receptors were found in* Staphylococcus aureus *but not in* Staphylococcus epidermidis, *a noninvasive pathogen. There were about 100 binding sites per cell, with an apparent binding affinity of 2.9 nanomolar. The molecular weight of the receptor was 50,000 and* pI *was 4.2. Eukaryotic laminin receptors were visualized by means of the binding of* S. aureus *in the presence of laminin. Prokaryotic and eukaryotic invasive cells might utilize similar, if not identical, mechanisms for invasion.*

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Adhesion is an important prerequisite for bacterial infectivity. Specific molecules are involved in bacterial adhesion at both the bacterial and host cell surfaces (*1*). The role of laminin, the major glycoprotein of the basement membrane, in the adhesion of pathogens such as sizing cancer cells (*5*), macrophages (*6*), and leukocytes (*7*).

Once a devastating pathogen, with over 80 percent mortality, *Staphylococcus aureus* is now the most common cause of severe infection in the nonimmunocompromised patient. In the industrialized world, it is responsible for endocarditis, osteomyelitis, arthritis, soft tissue infection, and pneumonia. In underdeveloped countries, staphylococcal infection is even more serious, with untreated disease often leading to fatal bacteremia (*8*).

Staphylococcus epidermidis is considered to be a nonpathogenic organism (*9*); it can, however, be an important pathogen when delivered to the actual site of

Laminin Receptors in *Staphylococcus aureus*

- *S. aureus* invasive pathogen
  Bloodstream invasion and widespread metastatic abscesses
- *S. epidermidis* non-invase

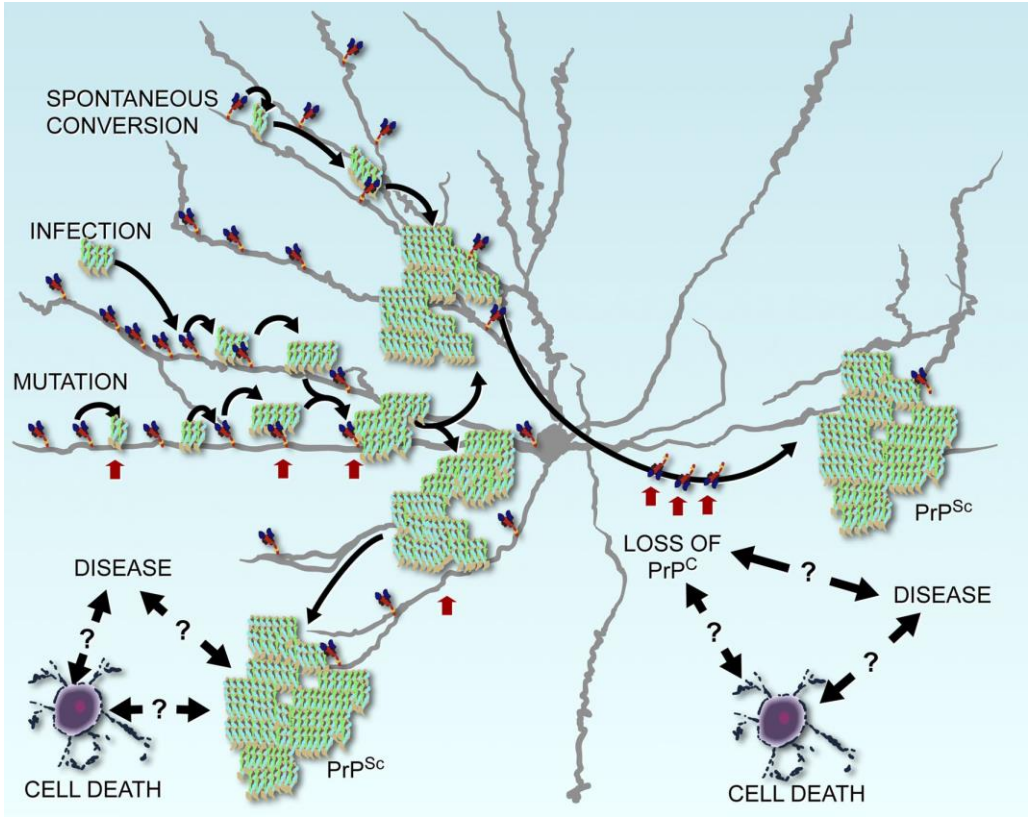
- *S. aureus*:
  100 binding sites for laminin per cell

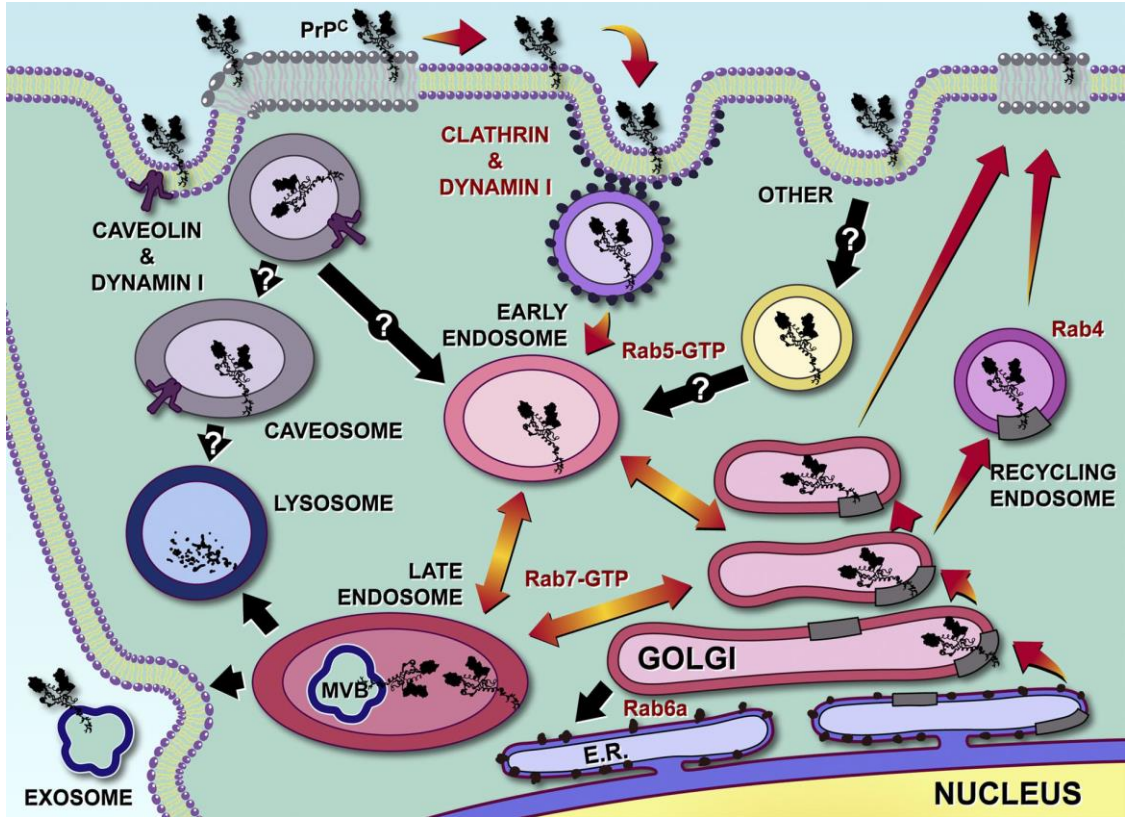
- *S. epidermidis*:
  No binding receptors for laminin

Physiology of the Prion Protein

RAFAEL LINDEN, VILMA R. MARTINS, MARCO A. M. PRADO, MARTÍN CAMMAROTA, IVÁN IZQUIERDO, AND RICARDO R. BRENTANI

Instituto de Biofísica da Universidade Federal do Rio de Janeiro, Rio de Janeiro; Ludwig Institute for Cancer Research, Hospital Alemão Oswaldo Cruz, São Paulo; Programa de Farmacologia Molecular, Universidade Federal de Minas Gerais, Belo Horizonte; Centro de Memória, Pontifica Universidade Católica do Rio Grande do Sul, Porto Alegre; Hospital A. C. Camargo and Faculdade de Medicina, University of São Paulo, São Paulo, Brazil



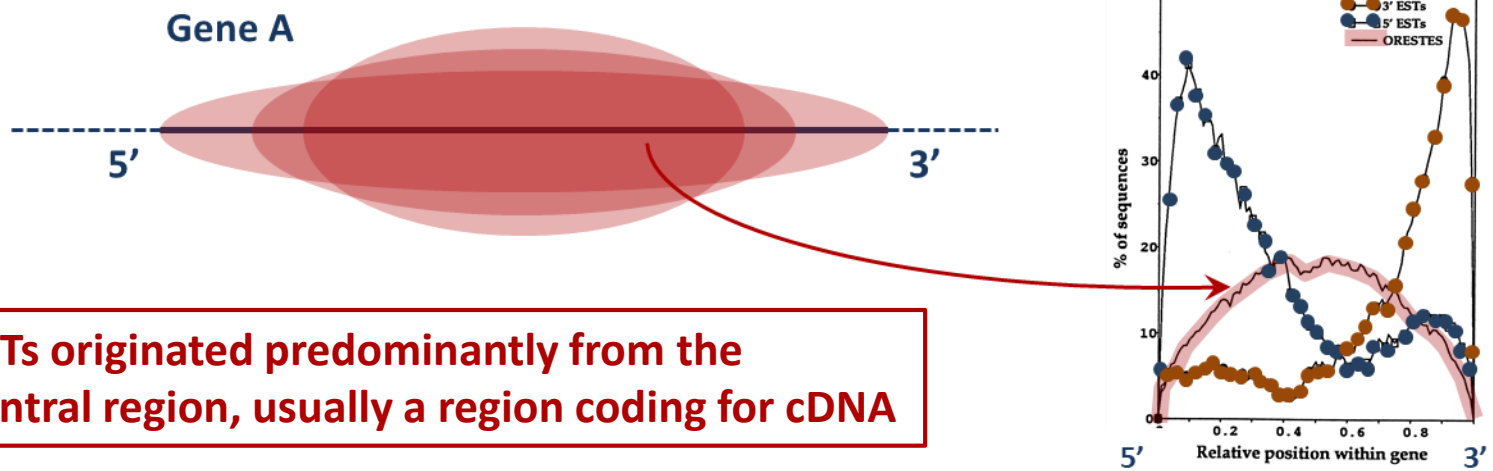


CANCER GENOME

ANALYSIS OF TRANSCRIPTOME
EST: expressed sequence tags
ORESTES: Open Reading (Frame) Expressed Sequence Tags

Manoel Dias-Neto
Andrew J Simpson

DNA
↓
RNA Transcripts
↓
Reverse Transciption PCR
↓
Sequences (*tags*) of specific genes



ESTs originated predominantly from the central region, usually a region coding for cDNA

CANCER GENOME

PROCEEDINGS OF THE NATIONAL ACADMY OF SCIENCES 2000

Shotgun sequencing of the human transcriptome with ORF expressed sequence tags

Emmanuel Dias Neto[a], Ricardo Garcia Correa[a], Sergio Verjovski-Almeida[b], Marcelo R. S. Briones[c], Maria Aparecida Nagai[d], Wilson da Silva, Jr.[e], Marco Antonio Zago[e], Silvana Bordin[f], Fernando Ferreira Costa[f], Gustavo Henrique Goldman[g], Alex F. Carvalho[a], Adriana Matsukuma[b], Gilson S. Baia[b], David H. Simpson[h], Adriana Brunstein[a], Paulo S. L. de Oliveira[a], Philipp Bucher[i], C. Victor Jongeneel[j], Michael J. O'Hare[k], Fernando Soares[l], Ricardo R. Brentani[a], Luis F. L. Reis[a], Sandro J. de Souza[a], and Andrew J. G. Simpson[a,m]

PROCEEDINGS OF THE NATIONAL ACADMY OF SCIENCES 2000

Identification of human chromosome 22 transcribed sequences with ORF expressed sequence tags

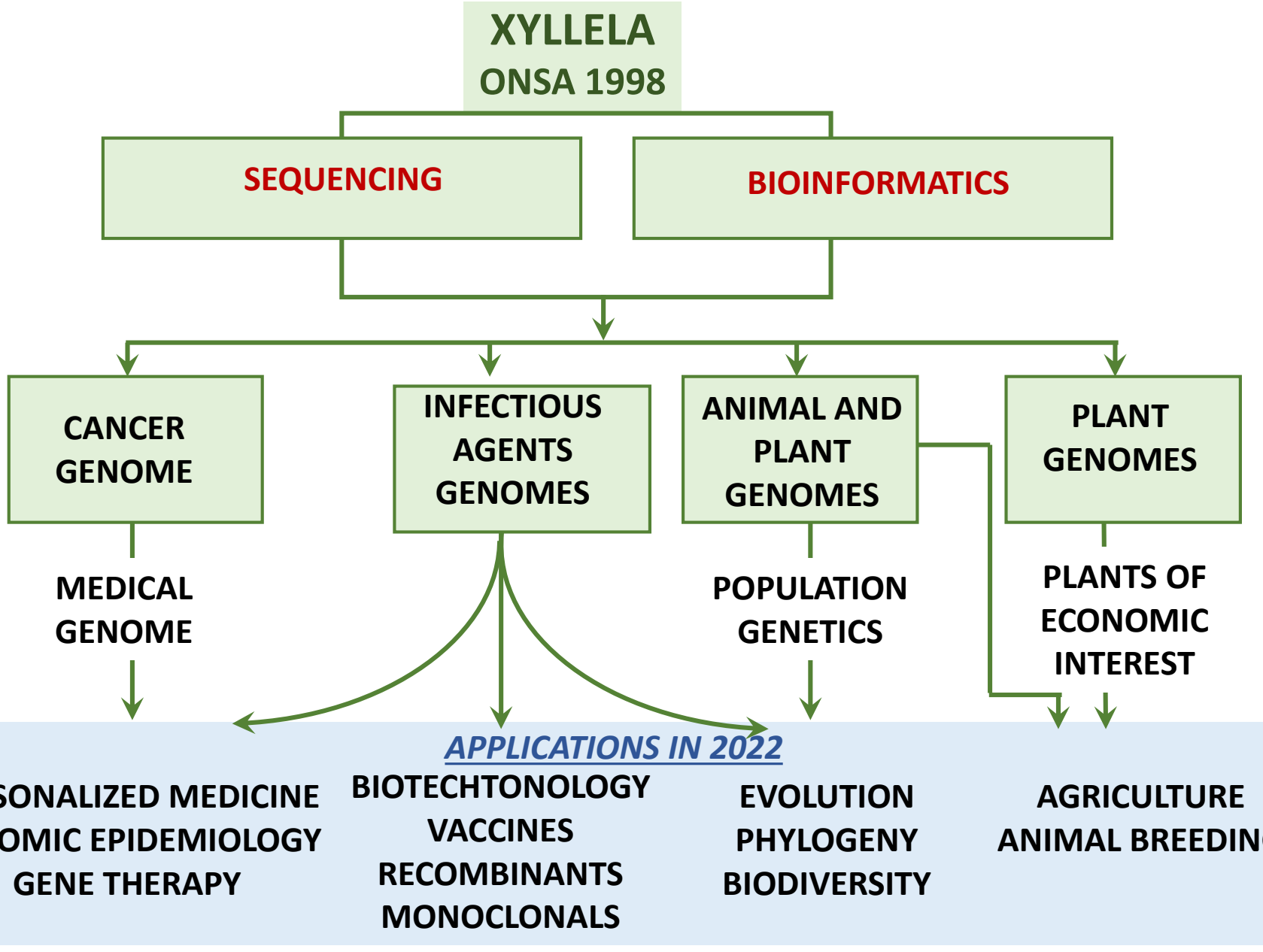
Sandro J. de Souza[a], Anamaria A. Camargo[a], Marcelo R. S. Briones[b], Fernando F. Costa[c], Maria Aparecida Nagai[d], Sergio Verjovski-Almeida[e], Marco A. Zago[f], Luis Eduardo C. Andrade[g], Helaine Carrer[h], Hamza F. A. El-Dorry[e], Enilza M. Espreafico[i], Angelita Habr-Gama[j], Daniel Giannella-Neto[k], Gustavo H. Goldman[l], Arthur Gruber[m], Christine Hackel[n], Edna T. Kimu
Maria Luisa Paçó-Larson[i], Maria
Silvia R. Rogatto[x], Ismael D. C. G
Sandro R. Valentini[bb], Marcio A
Mário Henrique Bengtson[e], Dirc
Maria Lucia C. Corrêa[k], Maria Cr
Luciana C. C. Leite[r], Gustavo Ma
Carlos Alberto Mestriner[bb], Elisa
Francisco G. Nóbrega[s], Élida P. B
Claudia A. Rainho[x], Nancy da Ro
Wilson da Silva, Jr.[f], Daniel F. Si
Heloisa Zalcberg[a], Ricardo R. Br

PROCEEDINGS OF THE NATIONAL ACADMY OF SCIENCES 2001

The contribution of 700,000 ORF sequence tags to the definition of the human transcriptome

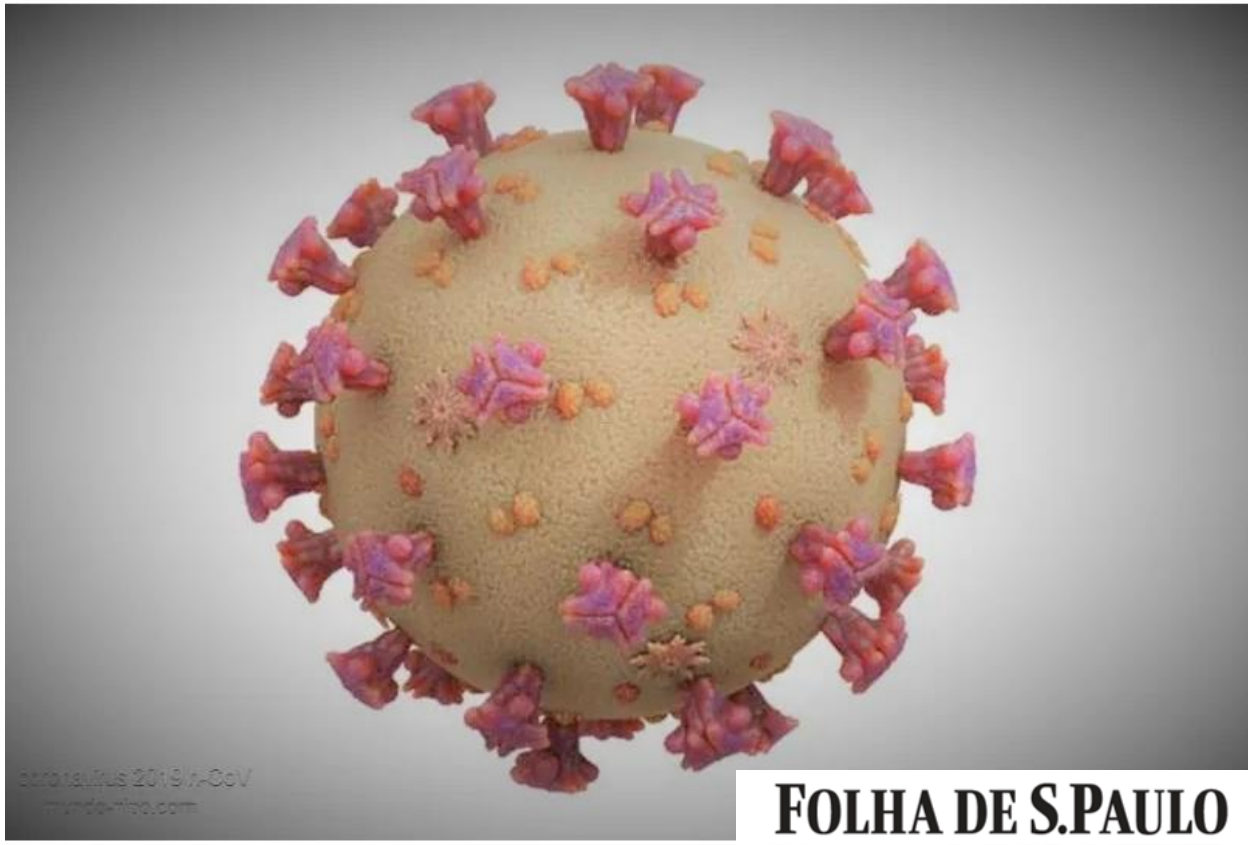
Anamaria A. Camargo[a], Helena P. B. Samaia[a], Emmanuel Dias-Neto[a], Daniel F. Simão[a], Italo A. Migotto[a], Marcelo R. S. Briones[b], Fernando F. Costa[c], Maria Aparecida Nagai[d], Sergio Verjovski-Almeida[e], Marco A. Zago[f], Luis Eduardo C. Andrade[g], Helaine Carrer[h], Hamza F. A. El-Dorry[e], Enilza M. Espreafico[i], Angelita Habr-Gama[j], Daniel Giannella-Neto[k], Gustavo H. Goldman[l], Arthur Gruber[m], Christine Hackel[n], Edna T. Kimura[o], Rui M. B. Maciel[p], Suely K. N. Marie[q], Elizabeth A. L. Martins[r], Marina P. Nóbrega[s], Maria Luisa Paçó-Larson[i], Maria Inês M. C. Pardini[t], Gonçalo G. Pereira[u], João Bosco Pesquero[v], Vanderlei Rodrigues[w], Silvia R. Rogatto[x], Ismael D. C. G. da Silva[y], Mari C. Sogayar[e], Maria de Fátima Sonati[z], Eloiza H. Tajara[aa], Sandro R. Valentini[bb], Fernando L. Alberto[c], Maria Elisabete J. Amaral[aa], Ivy Aneas[j], Liliane A. T. Arnaldi[p], Angela M. de Assis[c], Mário Henrique Bengtson[e], Nadia Aparecida Bergamo[x], Vanessa Bombonato[t], Maria E. R. de Camargo[n], Renata A. Canevari[x], Dirce M. Carraro[h], Janete M. Cerutti[p], Maria Lucia C. Corrêa[k], Rosana F. R. Corrêa[j], Maria Cristina R. Costa[f], Cyntia Curcio[o], Paula O. M. Hokama[t], Ari J. S. Ferreira[e], Gilberto K. Furuzawa[p], Tsieko Gushiken[t], Paulo L. Ho[r], Elza Kimura[z], José E. Krieger[i], Luciana C. C. Leite[r], Paromita Majumder[i], Mozart Marins[j], Everaldo R. Marques[j], Analy S. A. Melo[b], Monica Barbosa de Melo[c], Carlos Alberto Mestriner[bb], Elisabete C. Miracca[d], Daniela C. Miranda[m], Ana Lucia T. O. Nascimento[r], Francisco G. Nóbrega[s], Elida P. B. Ojopi[x], José Rodrigo C. Pandolfi[bb], Luciana G. Pessoa[y], Aline C. Prevedel[z], Paula Rahal[aa], Claudia A. Rainho[x], Eduardo M. R. Reis[e], Marcelo L. Ribeiro[n], Nancy da Rós[d], Renata G. de Sá[w], Magaly M. Sales[t], Simone Cristina Sant'anna[z], Mariana L. dos Santos[d], Aline M. da Silva[e], Neusa P. da Silva[g], Wilson A. Silva, Jr.[f], Rosana A. da Silveira[t], Josane F. Sousa[j], Daniella Stecconi[e], Fernando Tsukumo[u], Valéria Valente[j], Fernando Soares[cc], Eloisa S. Moreira[a], Diana N. Nunes[a], Ricardo G. Correa[a], Heloisa Zalcberg[a], Alex F. Carvalho[a], Luis F. L. Reis[a], Ricardo R. Brentani[a], Andrew J. G. Simpson[a,dd], and Sandro J. de Souza[a]

LEGACY OF THE FAPESP GENOME PROGRAM



Cientistas do Brasil sequenciam genoma do novo coronavírus em apenas 48 horas

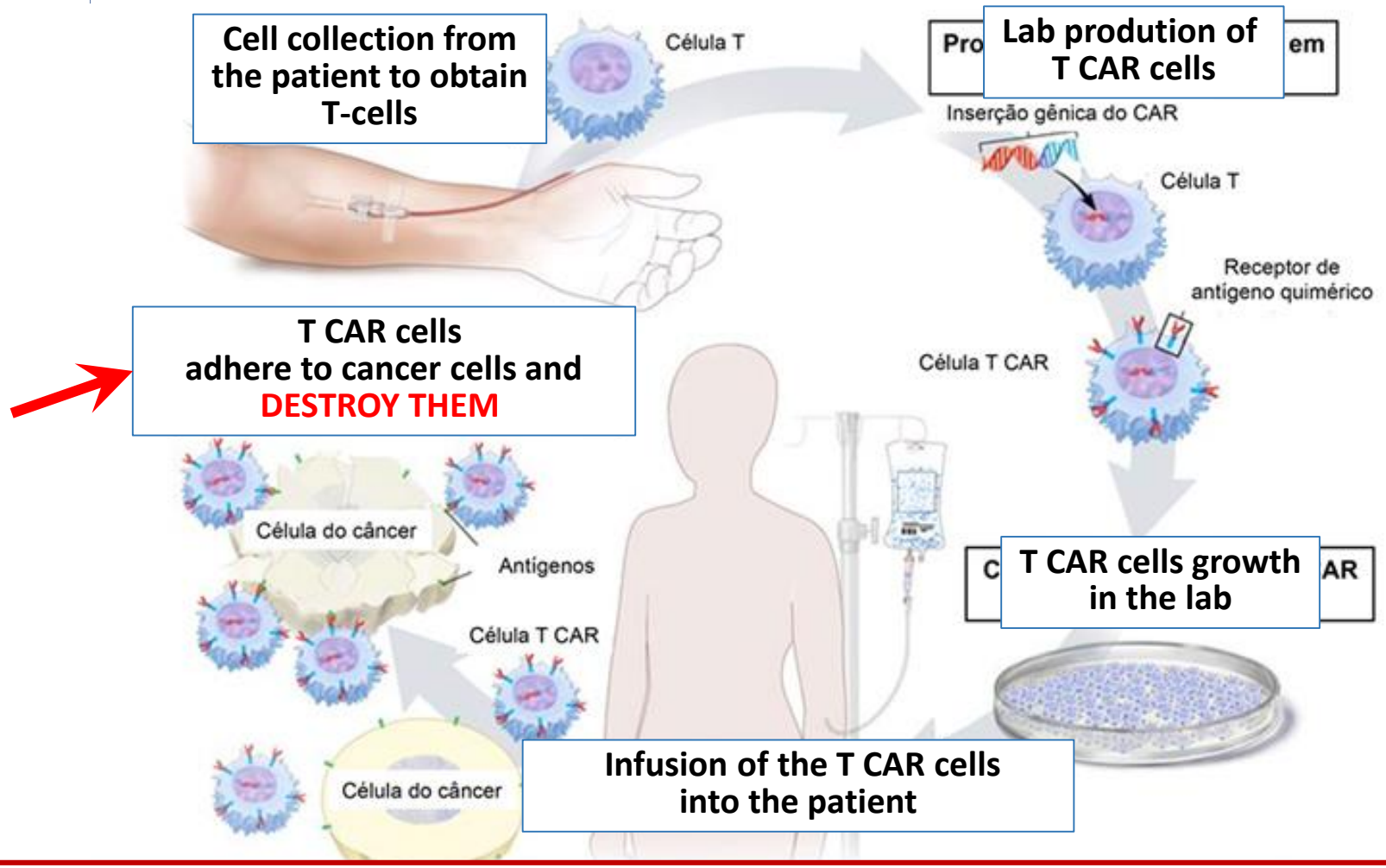
Brazilian scientists sequence the genome of the new coronavírus in just 48 hours



Brazil sequenced in 2 days, whereas others countries take an average of 15 days

INTERVENING IN THE GENOME

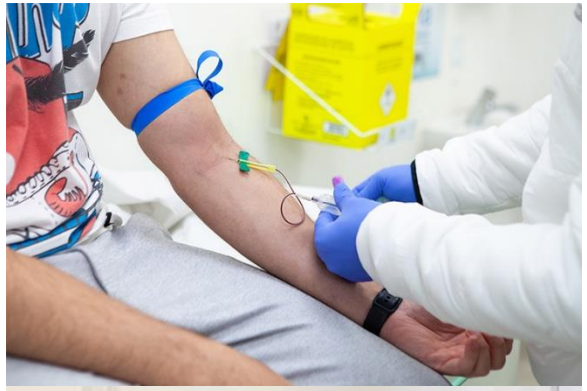
TREATMENT OF CANCER WITH T-CAR CELLS



ONLY EXAMPLE OF THIS FORM OF THERAPY IN LATIN AMERICA – FAPESP SUPPORTED CEPID IN RIBEIRÃO PRETO

Manipulating the genome
CAR T-CELL TERAPY FOR CANCER

7 patients treated in Brazil at the CEPID FAPESP



CENTER OF CELL THERAPY

UNIVERSITY HOSPITAL DE RIBEIRÃO PRETO

FAPESP 60 ANOS 1962 - 2022

GENOME 20+2

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1937 – 2011