



UNA TERAPIA MOLECULAR ALTERNATIVA PARA LA PROGERIA

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SINDROMES PROGEROIDES

Dermopatía restrictiva (RD) – pre-lámina A



- Retraso del crecimiento intrauterino
- Boca abierta
- Micrognacia
- Piel translúcida y delgada
- Lesiones esclerodermiformes
- Hipoplasia pulmonar
- Osteolisis de las falanges distales

ZMPSTE24

Dos mutaciones nulas

OMIM 275210

[141] [142]

Síndrome de Progeria atípico (APS)



- Retraso en el crecimiento
- Proptosis
- Micrognacia
- Apiñamiento dental
- Alopecia
- Osteolisis de las falanges distales
- Osteopenia
- Lipodistrofia

LMNA

c.1583C>T, p.T528M
and
c.1619T>C, p.M540T

[180]

Síndrome de Progeria de Nestor-Guillermo (NHPS)



- Retraso en el crecimiento
- Lipoatrofia generalizada
- Piel seca atrófica con defectos de pigmentación
- Apiñamiento dental
- Osteoporosis
- Deformaciones esqueléticas

BANF1

c.34G>A, p.A12T
Homocigoto

OMIM 614008

[143] [181]

Síndrome de Hallermann-Streiff (HSS)



- Braquicefalia con abombamiento frontal
- Hipotricosis
- Cataratas y microftalmia
- Micrognacia
- Anomalías dentales
- Corta estatura

Gen desconocido

OMIM 234100

Hennekam R.C.M, courtesy

EL SINDROME DE PROGERIA HUTCHINSON—GILFORD (HGPS)

Desorden genético raro (1/8 millones) originado por una mutación espontánea en el gen LMNA en el cromosoma 1.

Fenotipo: baja estatura, alopecia. piel delgada con manchas,, osteoporosis, arterosclerosis, miopía y articulaciones rígidas.

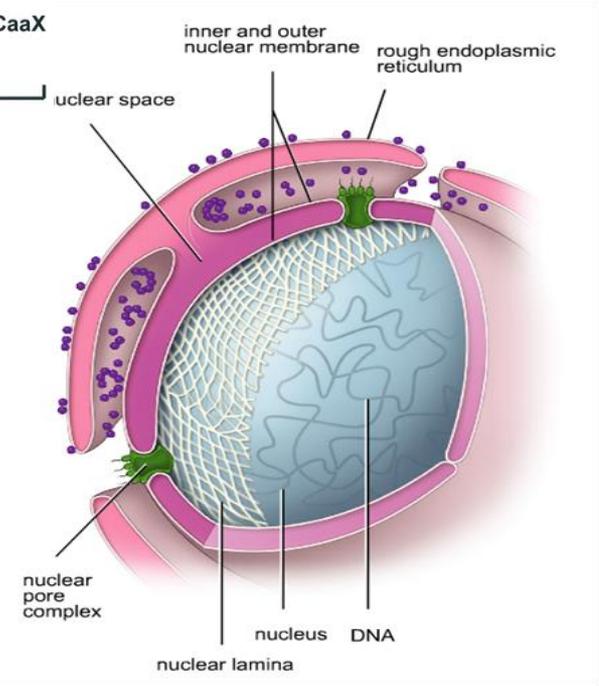
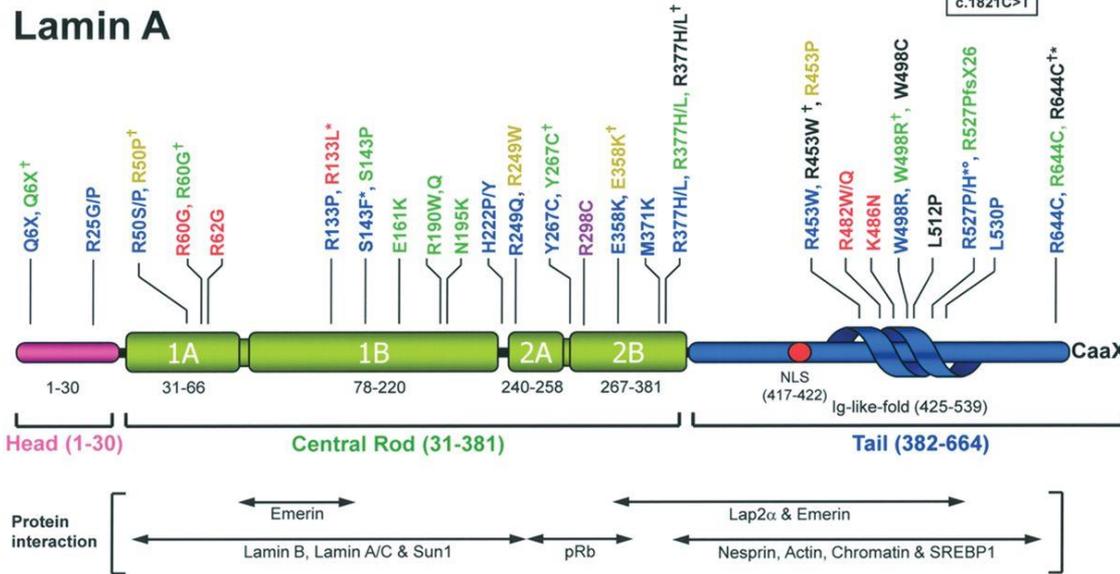
Expectativa de vida es de 15 años. Muerte por accidentes cerebro vasculares e infarto al miocardio



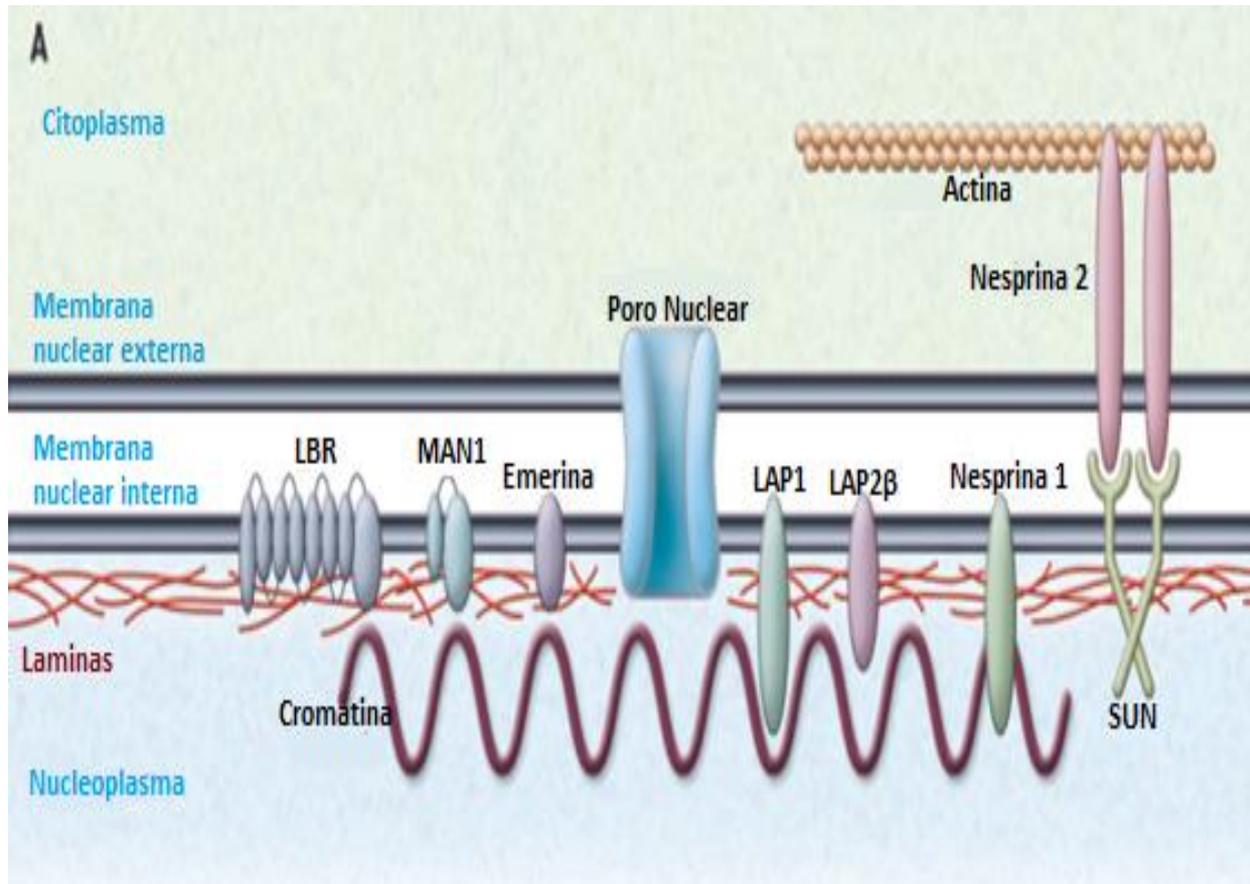
LA LAMINA NUCLEAR



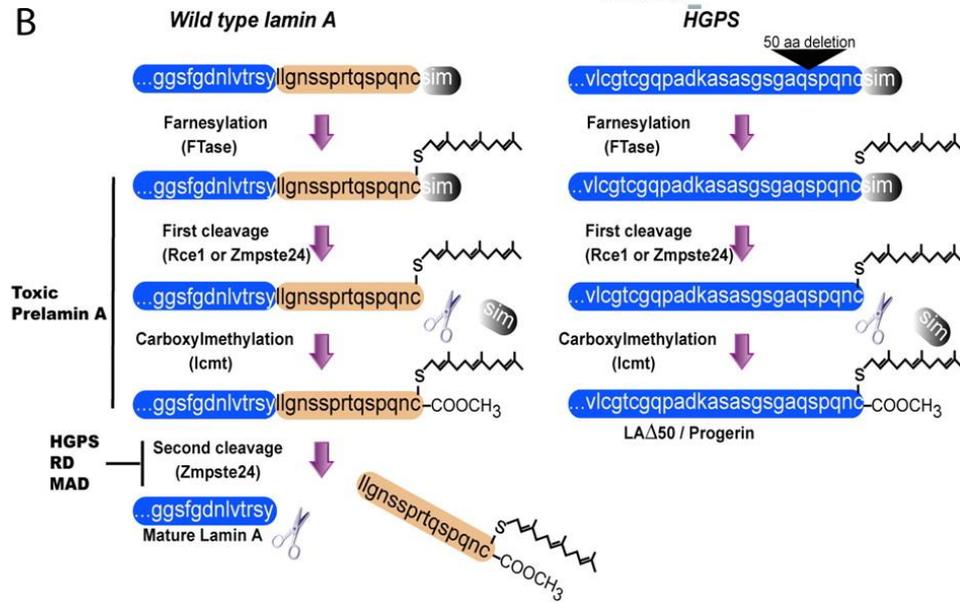
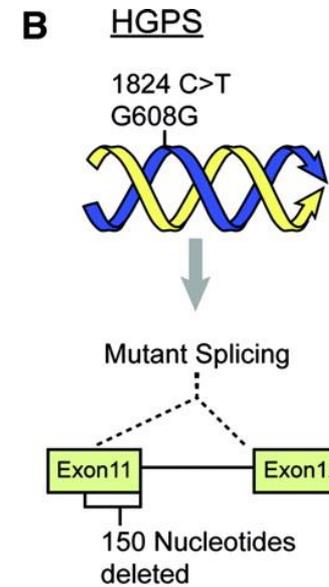
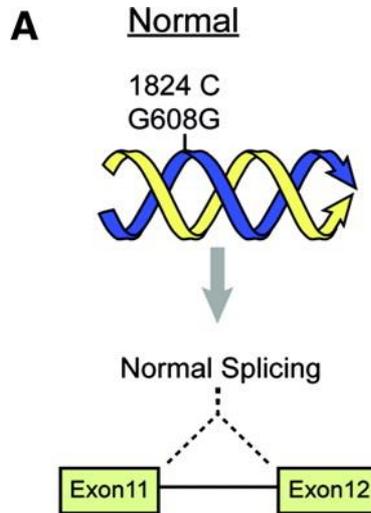
b) **Lamin A**



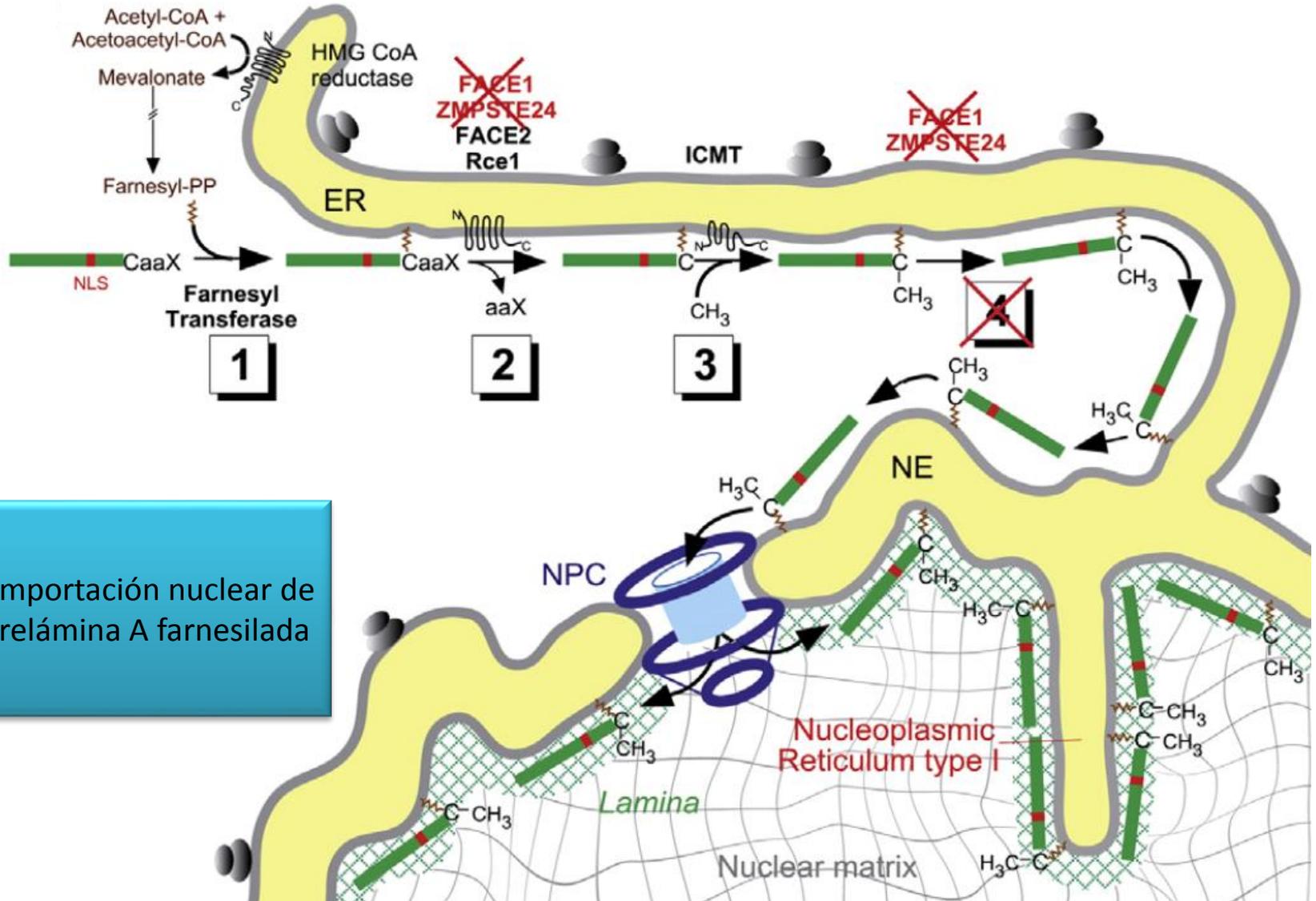
ORGANIZACIÓN DE LA ENVOLTURA NUCLEAR



BASES MOLECULARES DE LA PROGERIA



PROCESAMIENTO E IMPORTE NUCLEAR DE LA PROGERINA



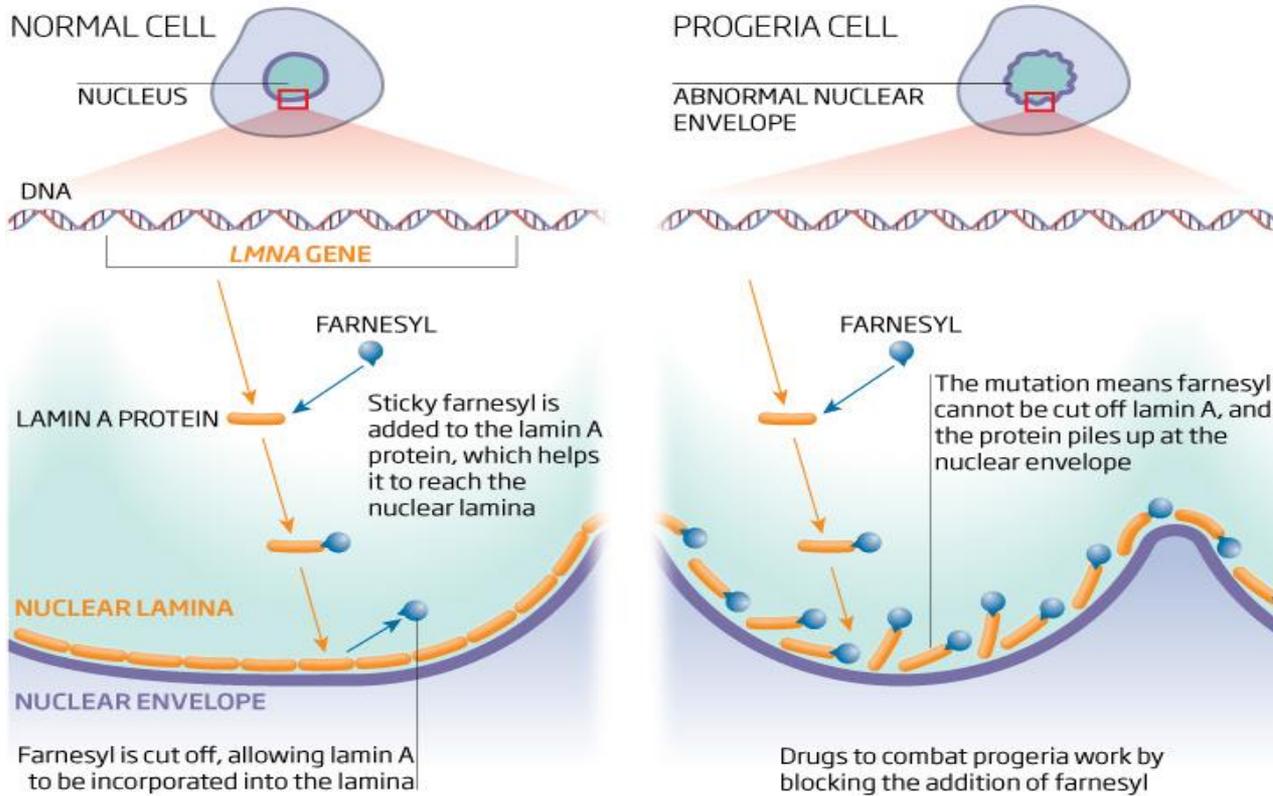
“Importación nuclear de prelámina A farnesilada”

EFECTO DOMINANTE DE LA PROGERINA

Ageing mutation

©NewScientist

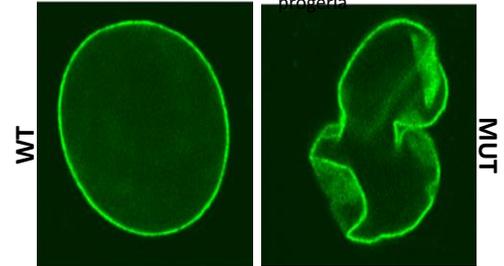
Progeria is a rare genetic condition in which children appear to age prematurely. The mutation is in the *LMNA* gene, which codes for a protein called lamin A. This acts as a scaffolding on the inner side of the cell nucleus



Immunofluorescencia de la lámina A/C

Núcleo de una célula de un individuo sano

Núcleo de una célula de un paciente con progeria

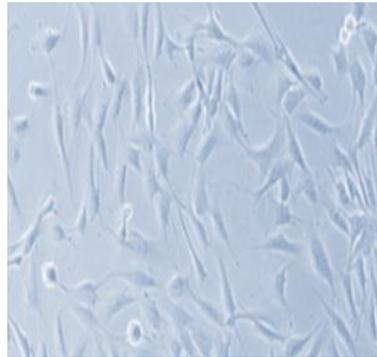


Scaffidi P, Gordon L, Misteli T (2005)

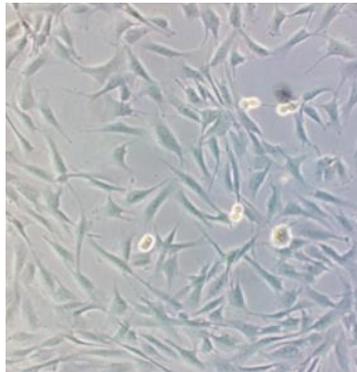
MODELO CELULAR PARA EL ESTUDIO DE EL HGPS

- **FIBROBLASTOS DE PIEL DE PACIENTES CON HGPS**

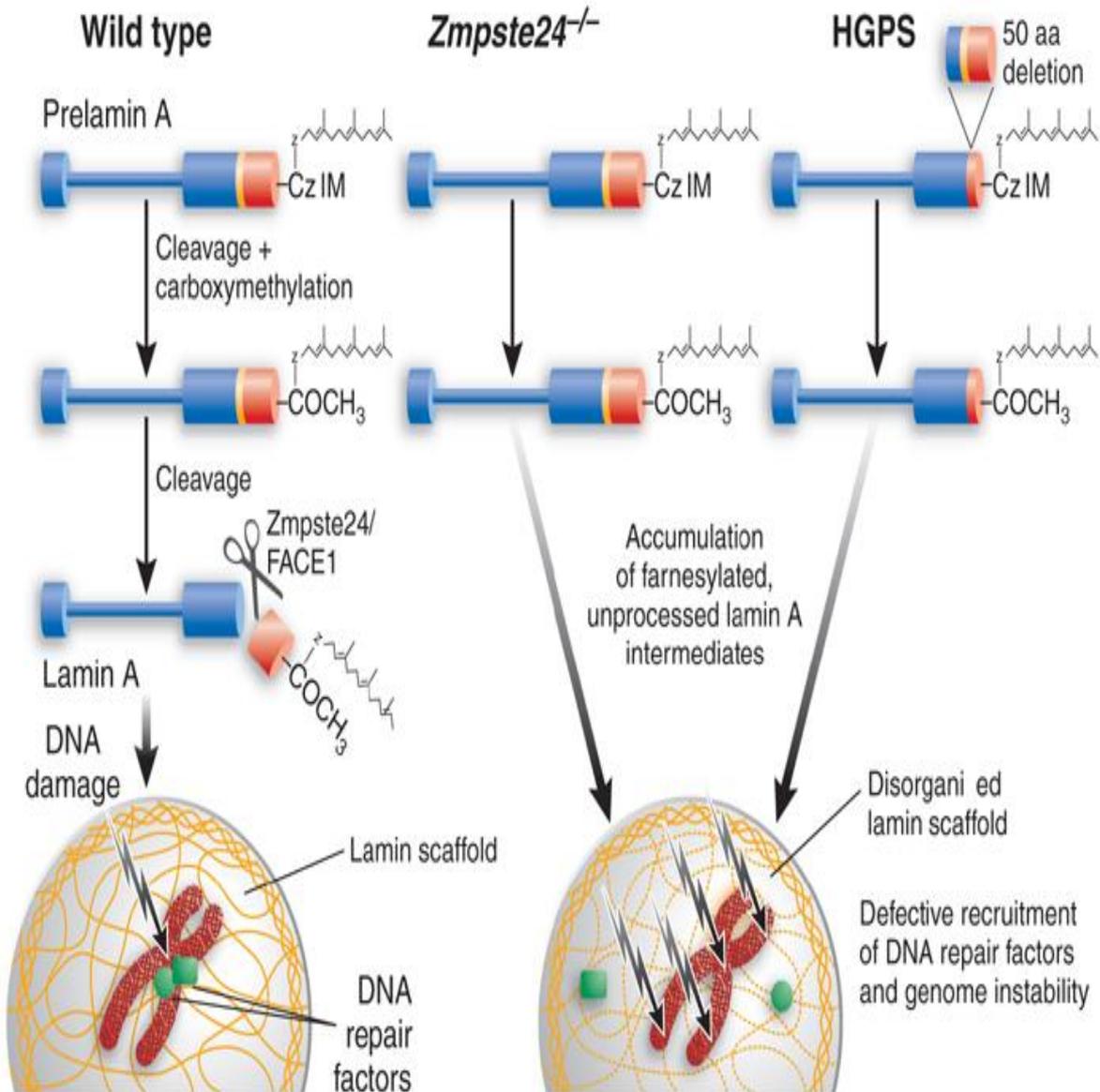
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- **FIBROBLASTOS DE PIEL DE INDIVIDUOS NORMALES**

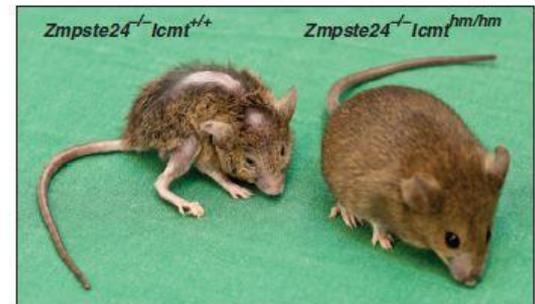


MODELOS ANIMALES PARA EL HGPS

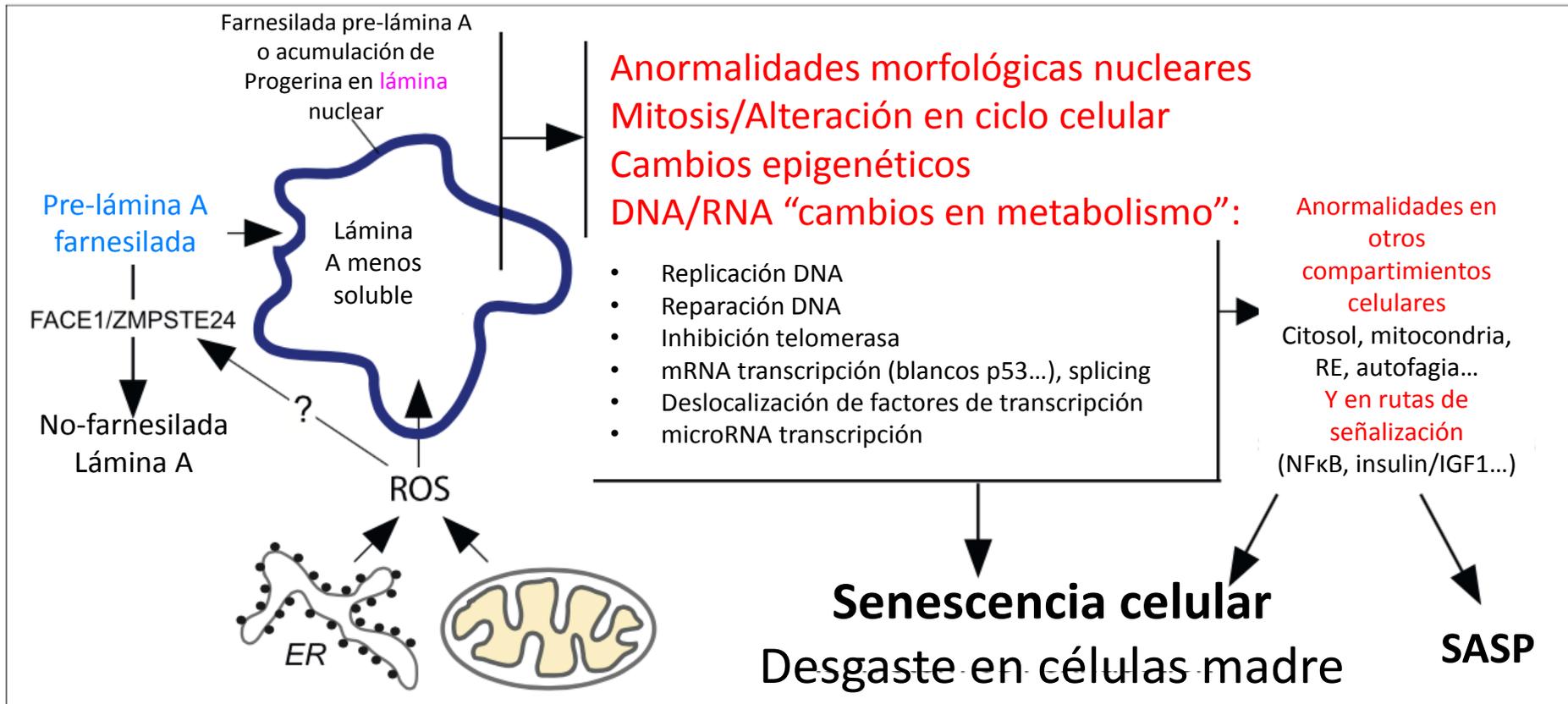


healthy mouse progeria mouse mouse treated with IGF1

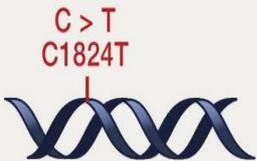
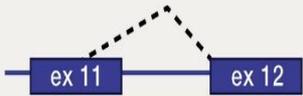
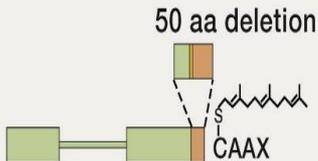
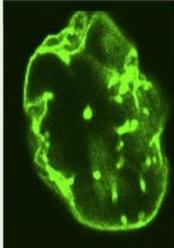
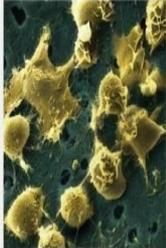
A



ALTERACIONES MOLECULARES DEL HGPS



“ESTRATEGIAS TERAPEUTICAS PARA ALIVIAR LA PROGERIA”

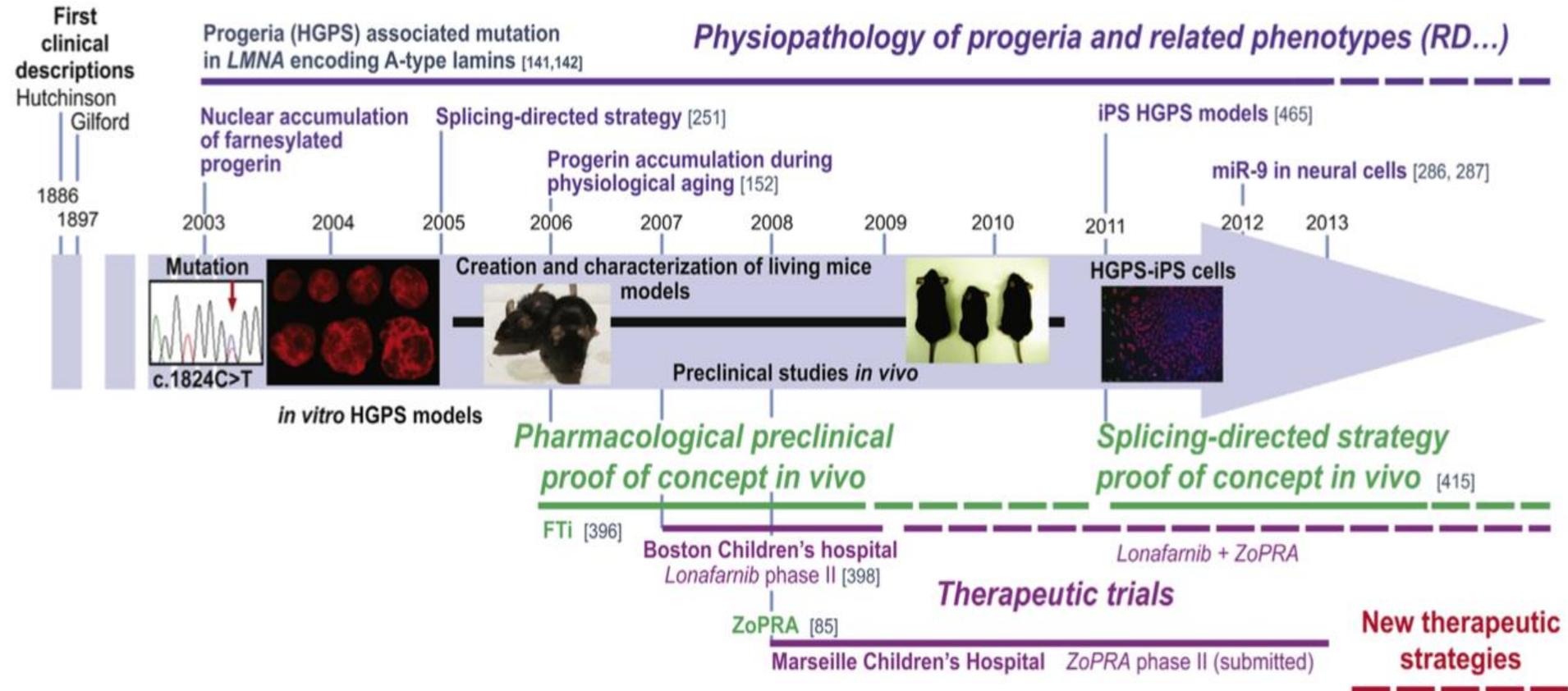
| DNA | RNA | Protein | Cell | Tissue |
|--|--|--|--|---|
|  <p>C > T C1824T</p> |  <p>ex 11 ex 12</p> |  <p>50 aa deletion CAAX</p> |  |  |
| <p>Gene correction Zn-fingers TALENs CRISPR</p> | <p>Pre-mRNA splicing correction Antisense approaches Small molecules</p> <p>RNA elimination shRNA RNase H-mediated degradation</p> | <p>Progerin correction FTIs Statins, bisphosphonates</p> <p>Progerin function Resveratrol</p> <p>Progerin turnover Rapamycin</p> | <p>Reversal of cellular defects Epigenetic reprogramming Anti-inflammatories</p> | <p>Cell replacement therapies</p> |
| <p>TREATMENT SPECIFICITY</p> | | | | |

“LOS ENSAYOS CLINICOS EN PACIENTES CON HGPS HAN FRACASADO”

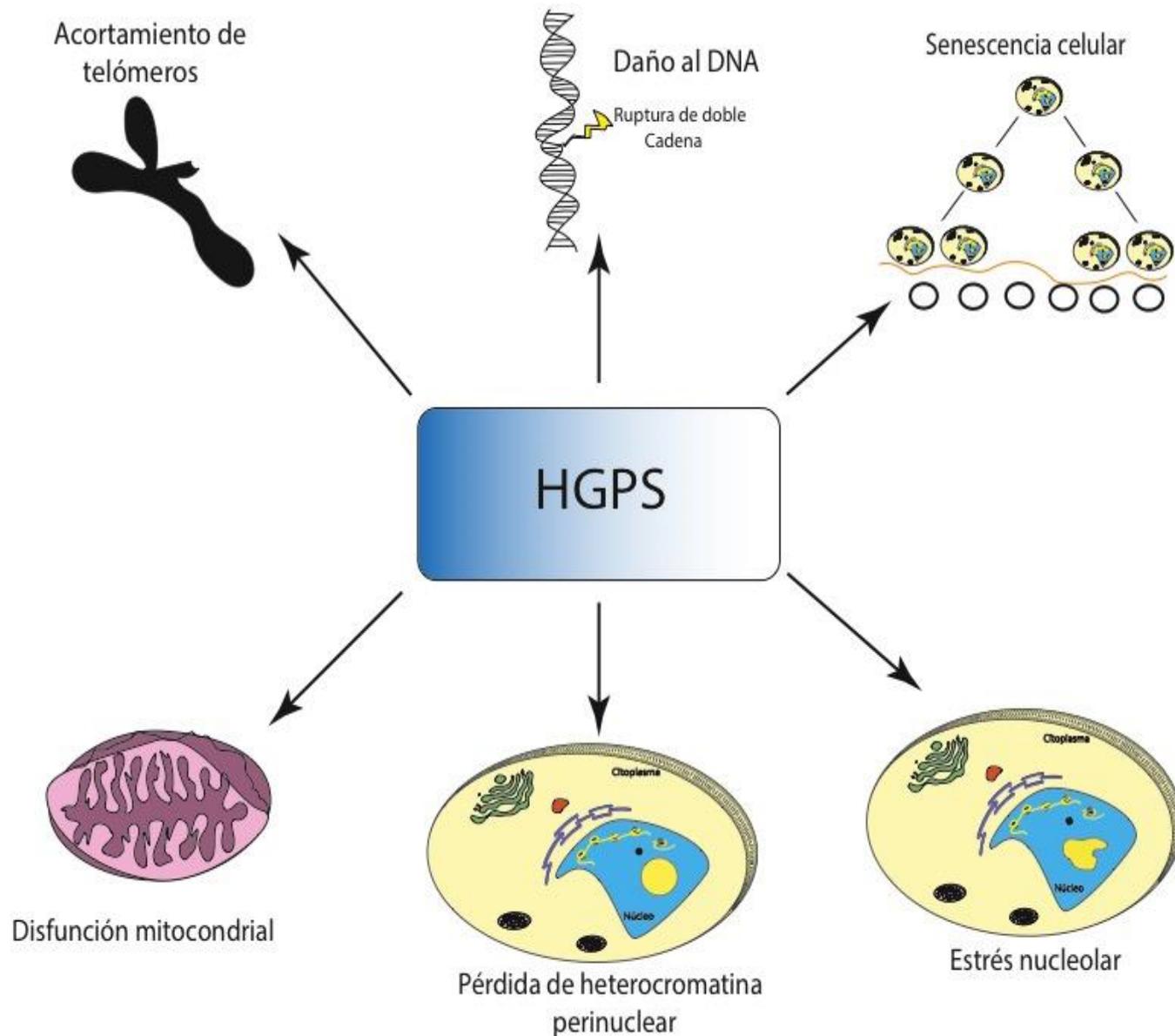
| Droga | Farmacodinamia | Efecto |
|---|---|---|
| <ul style="list-style-type: none">• 25 pacientes tratados con lonafarnib | <ul style="list-style-type: none">• Inhibidor de la farnesiltransferasa. | <ul style="list-style-type: none">• Modesta ganancia de peso.• Disminución leve de la rigidez arterial.• Aumento en la esperanza de vida de 1.6 años. |
| <ul style="list-style-type: none">• 45 pacientes tratados con lonafarnib y estatina. | <ul style="list-style-type: none">• Inhibidores de la farnesiltransferasa | <ul style="list-style-type: none">• Ensayos en curso. |



“10 años de investigación en progeria y en síndromes relacionados”



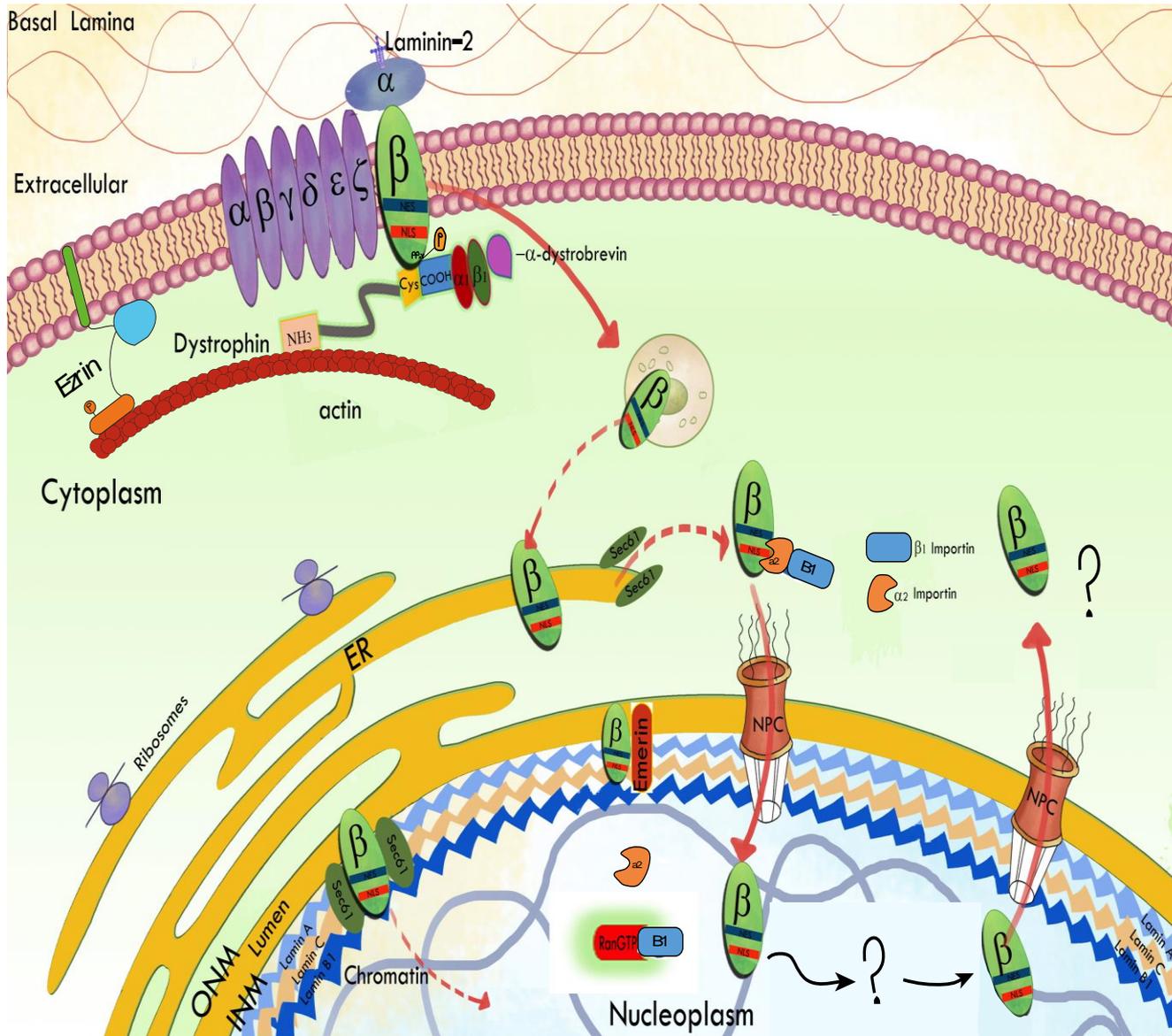
¿EXISTIE UN MECANISMO COMÚN PARA LAS ALTERACIONES MOLECULARES DE LA PROGERIA?



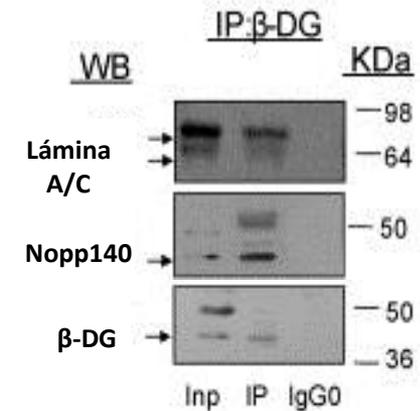
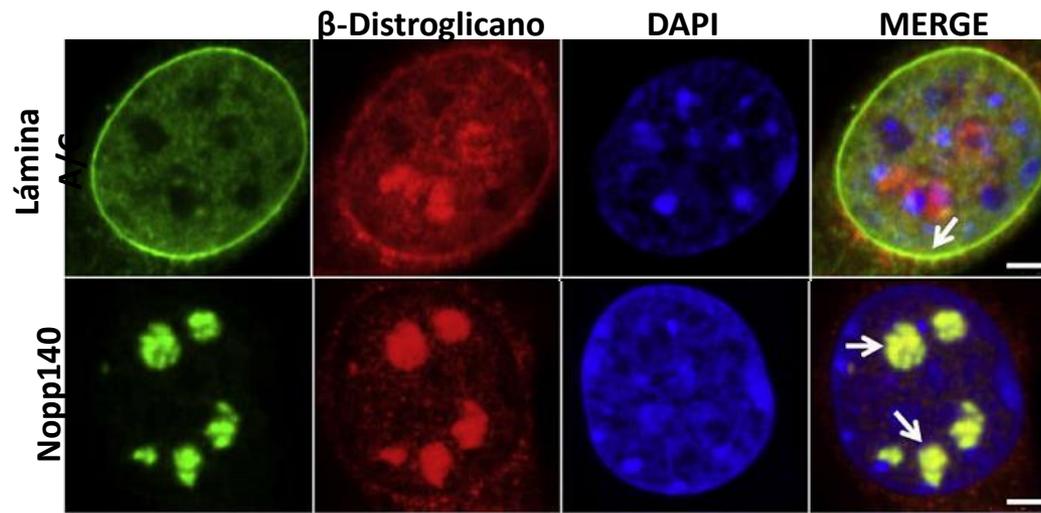


- **EL HALLAZGO DE UNA NUEVA PROTEÍNA IMPLICADA EN EL HGPS NOS LLEVO A LA IDENTIFICACIÓN DE UN BLANCO TERAPEÚTICO**

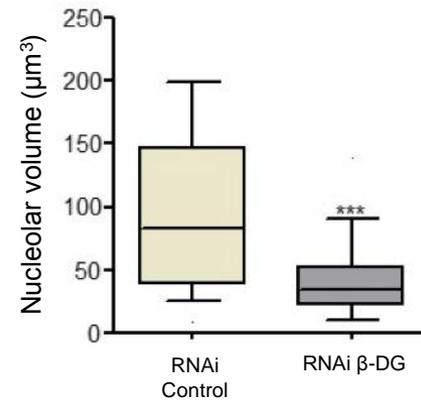
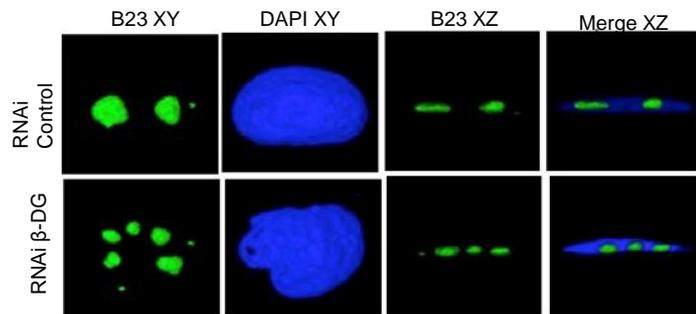
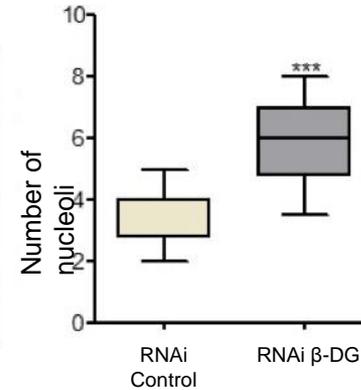
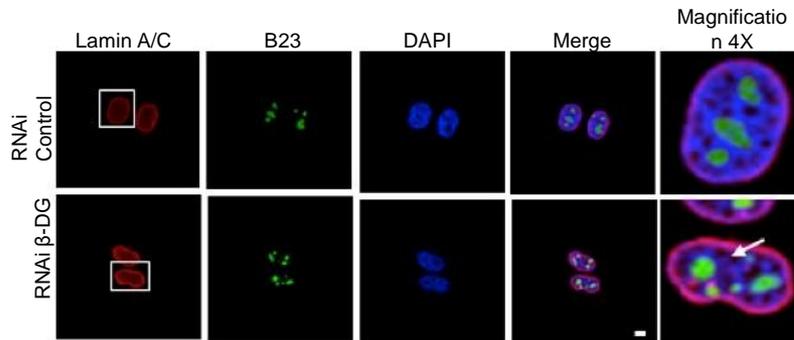
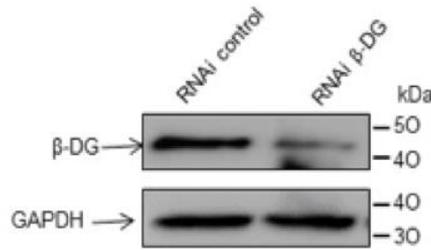
EL β -DG UNA NUEVA PROTEÍNA IMPLICADA EN LA PROGERIA



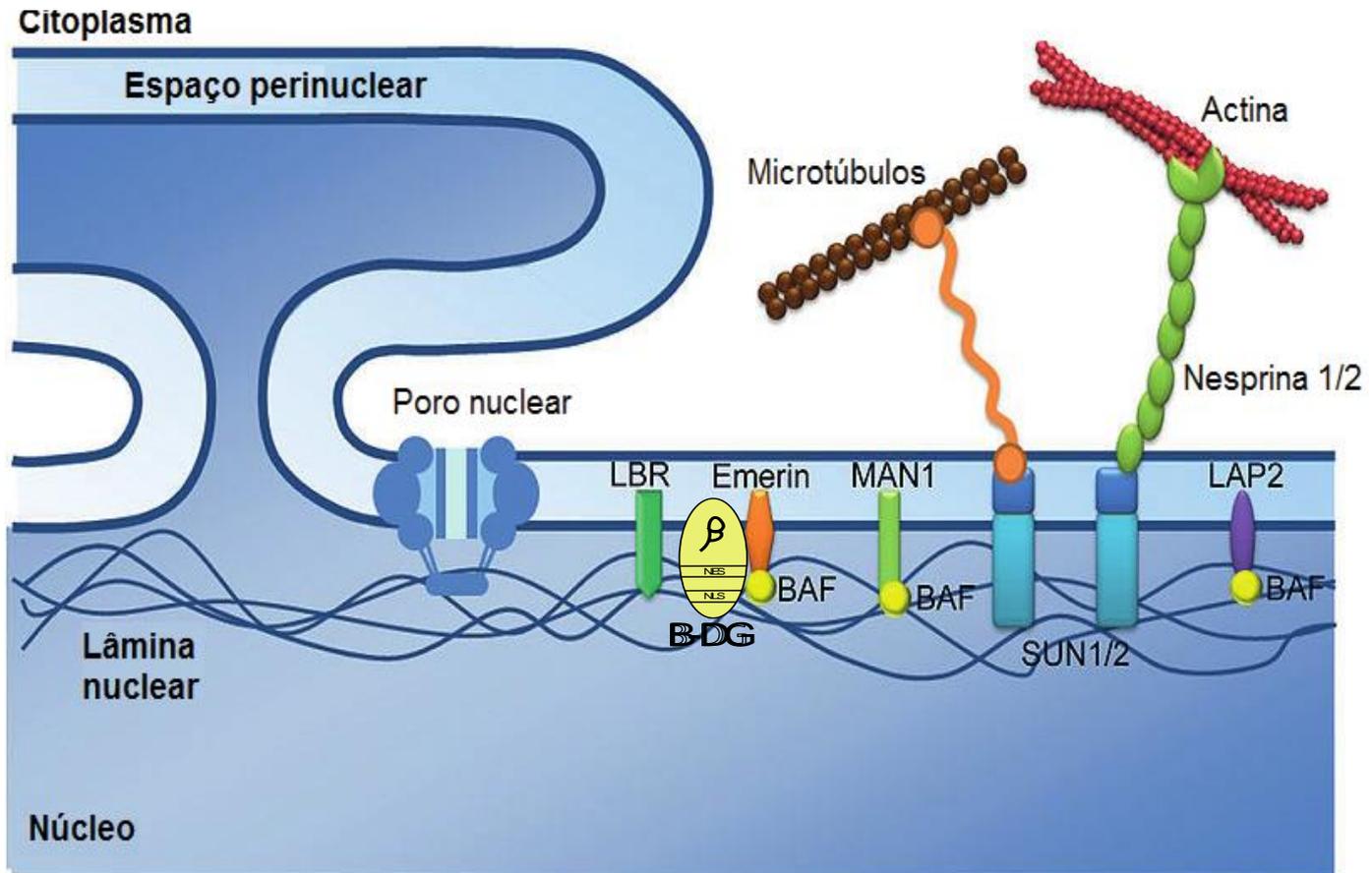
EL β -DG COLOCALIZA E INTERACCIONA CON LA LAMINA A EN LA ENVOLTURA NUCLEAR Y CON PROTEÍNAS NUCLEOLARES



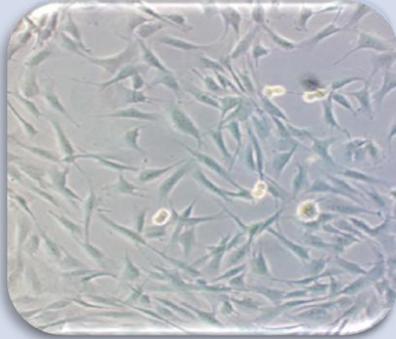
EL KNOCKDOWN DEL β -DG RECREA CARACTERISTICAS DE LA PROGERIA



EL B-DG ESTA IMPLICADO EN EL FENOTIPO DEL HGPS?



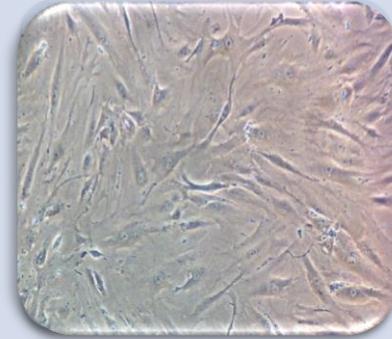
Modelo de estudio



**Células
AG0309:**
Fibroblastos
persona de
sexo femenino
caucásica sana.

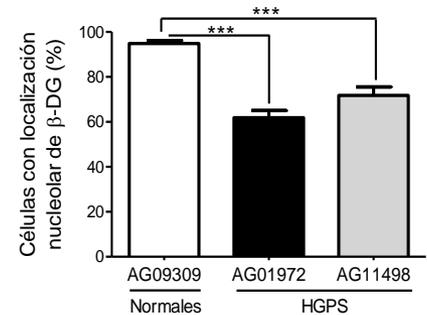
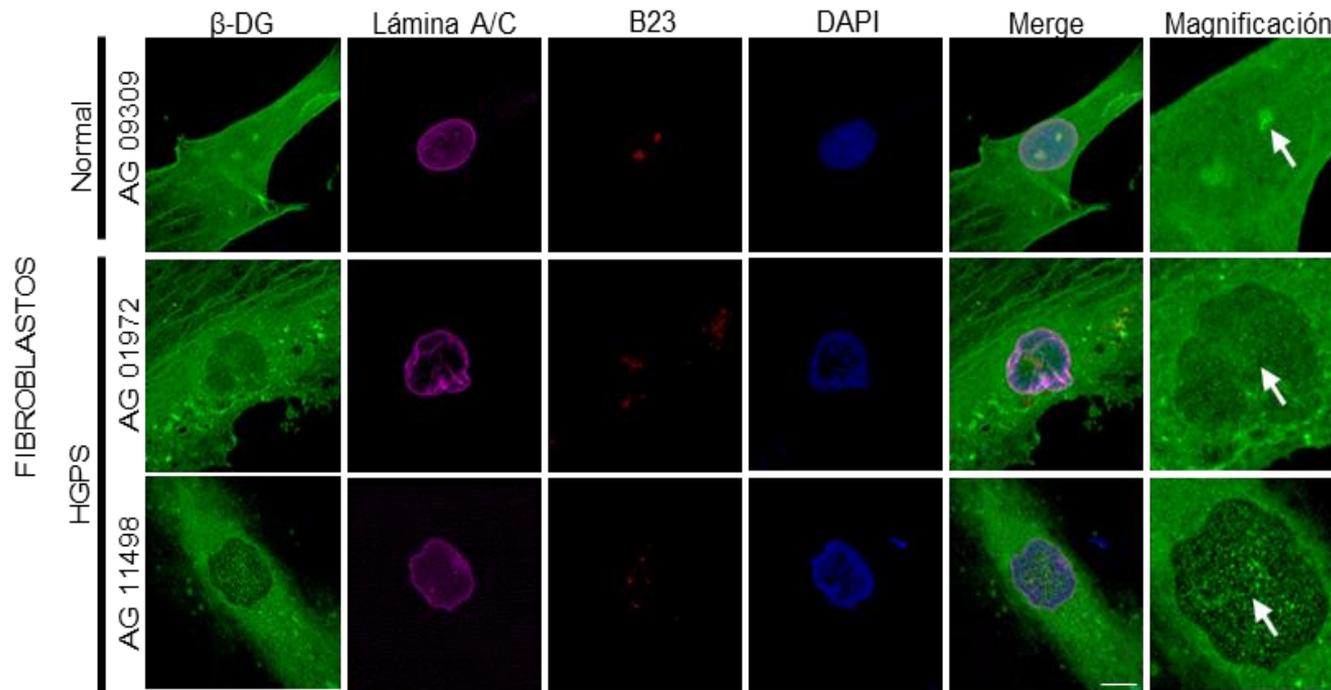


**Células
AG01972:**
Fibroblastos
paciente de
sexo femenino
caucásica con
Progeria.



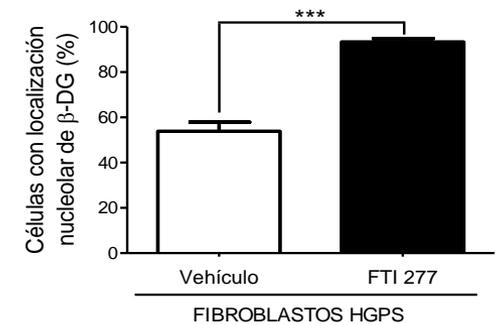
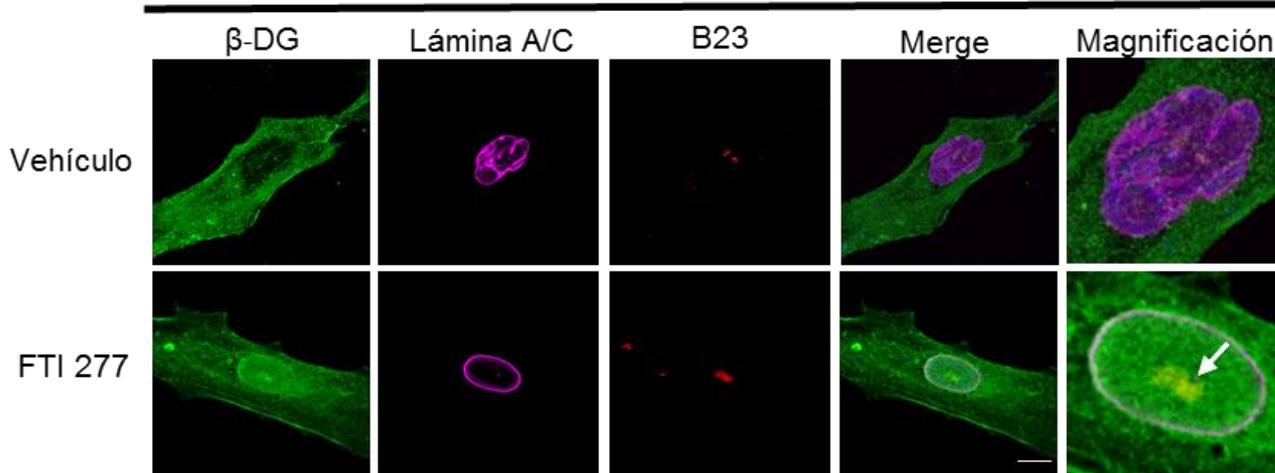
**Células
AG11498:**
Fibroblastos
paciente de
sexo masculino
con Progeria
con daño
cardiovascular.

La localización nuclear/nucleolar del β -DG está alterada en los fibroblastos de individuos con progeria

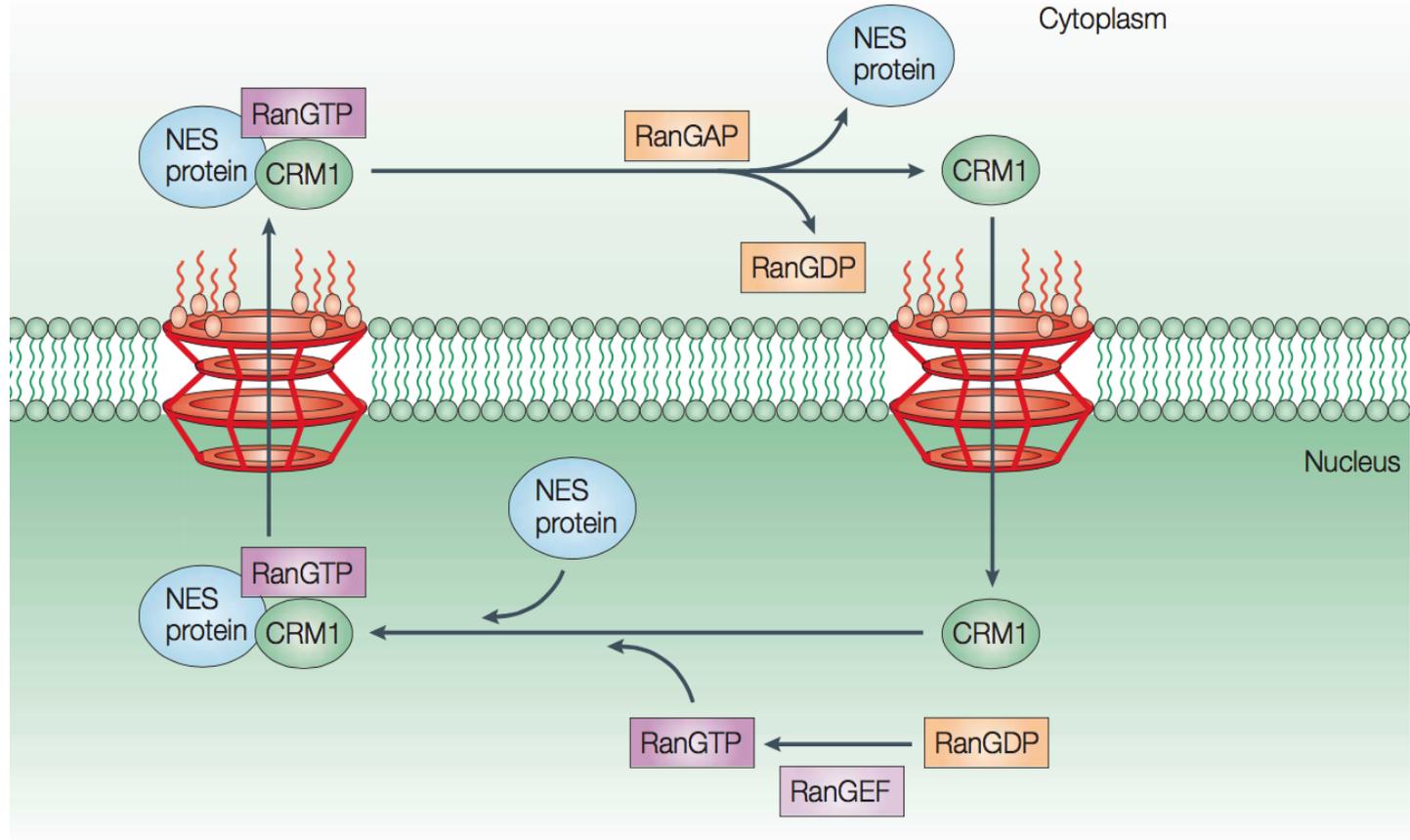
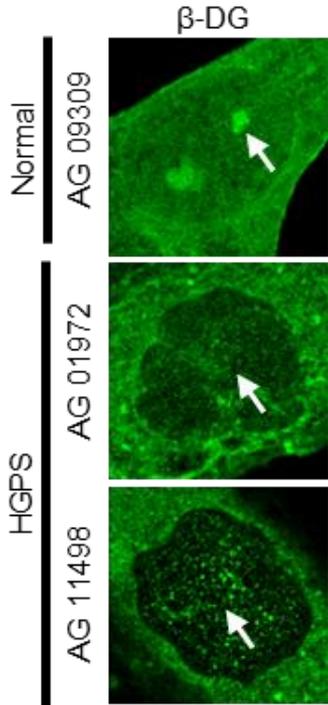


LA INHIBICION DE LA FARNESILACION DE LA PROGERINA CORRIGE LA MORFOLOGIA NUCLEAR Y RESTAURA LA LOCALIZACIÓN NUCLEOLAR DEL β -DG

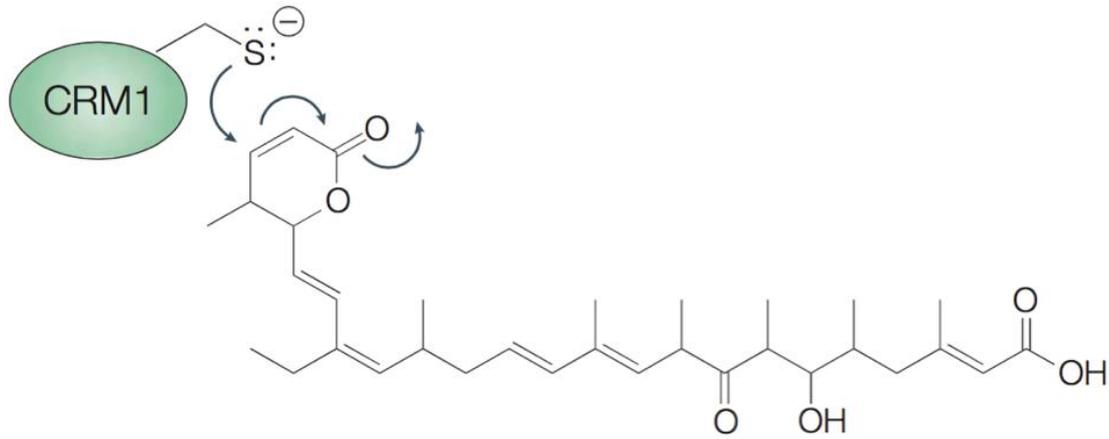
FIBROBLASTOS HGPS



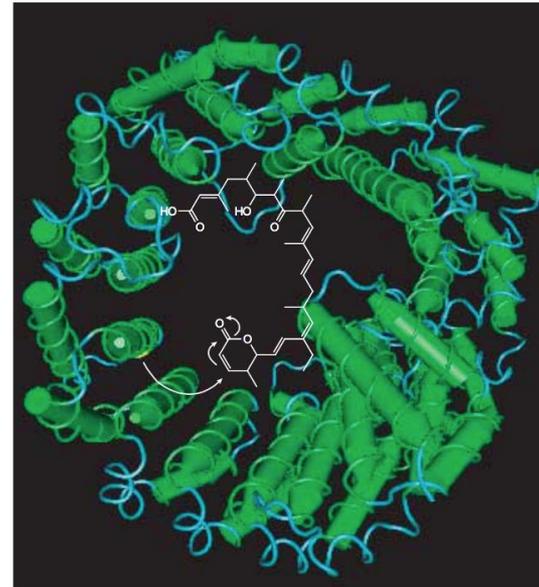
EL EXPORTE NUCLEAR DEL β -DG ESTA ALTERADO EN LAS CÉLULAS HGPS?



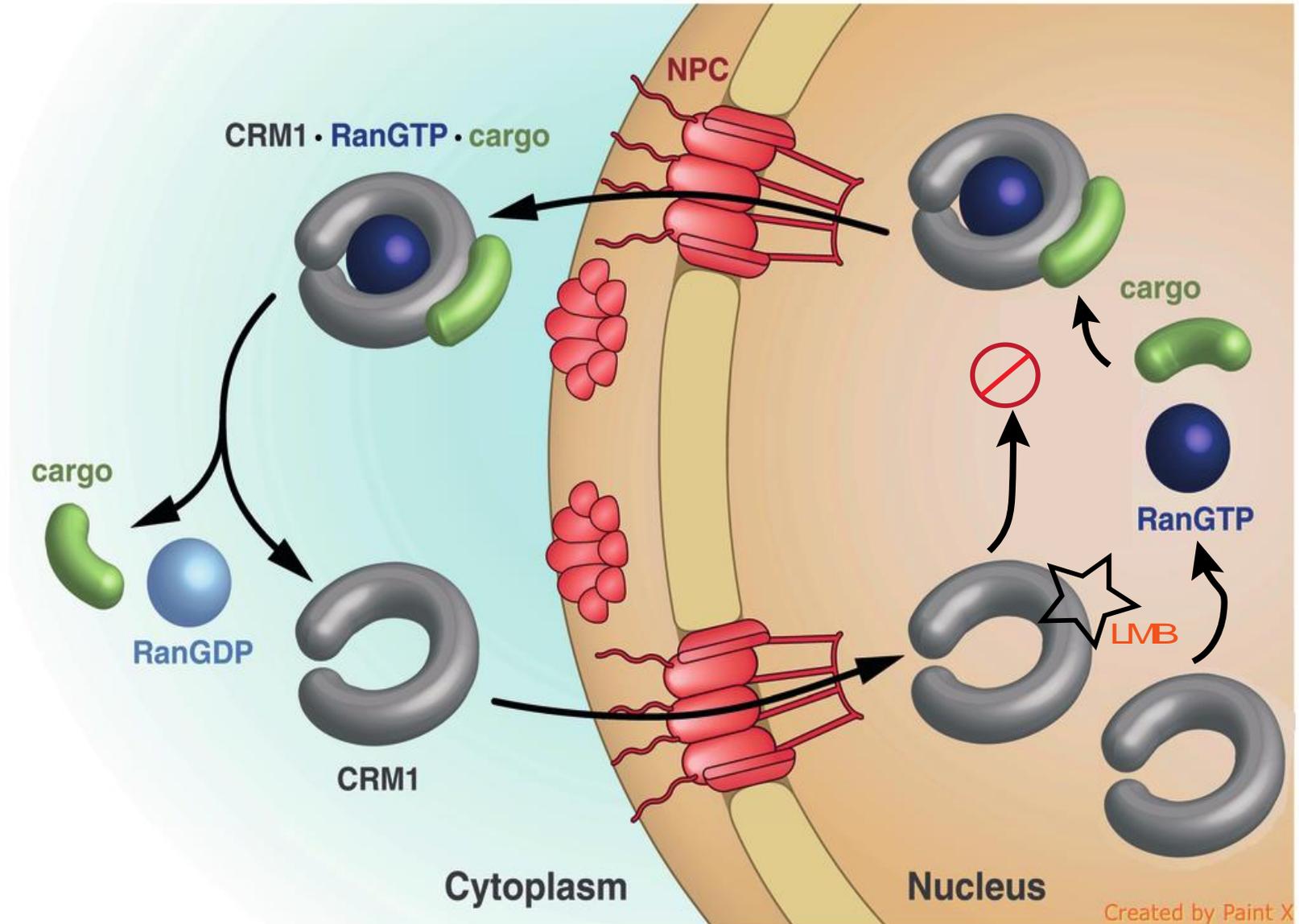
MECANISMO DE ACCIÓN DE LA LEPTOMICINA B



Leptomycin B

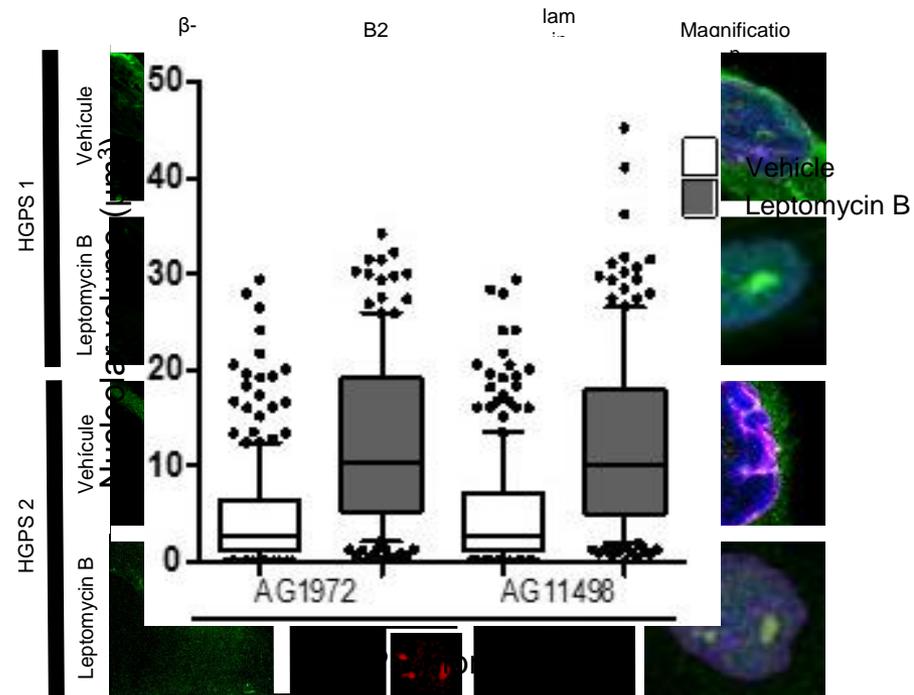


“EXPORTE NUCLEAR DE PROTEÍNAS MEDIADO POR CRM1”



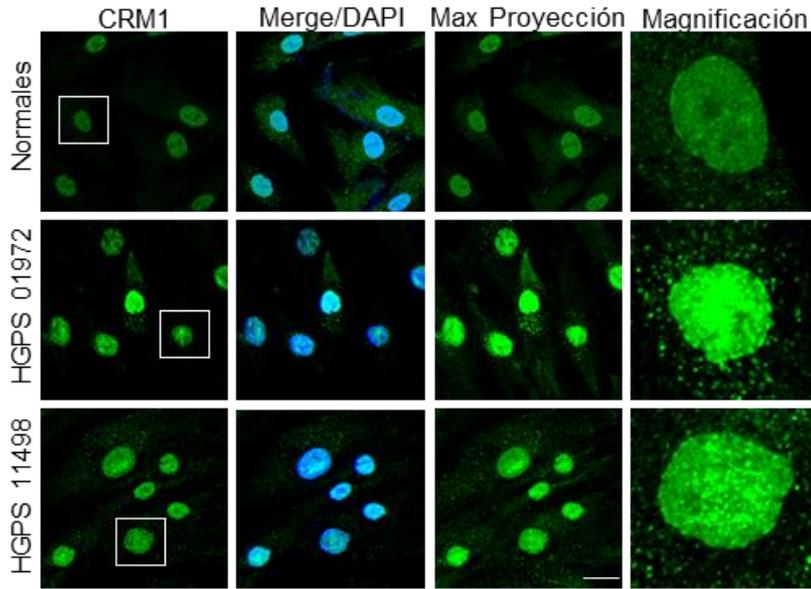
LA INHIBICION DEL EXPORTE NUCLEAR RESTAURA LA LOCALIZACION NUCLEOLAR DEL β -D₂ Y ALIVIA EL ESTRES NUCLEOLAR

A

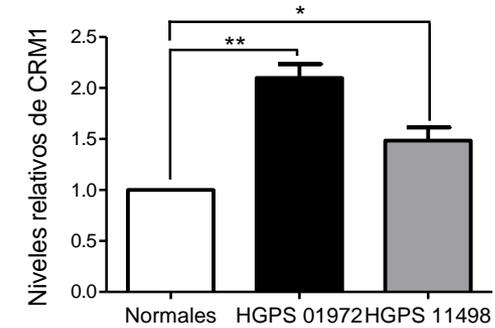
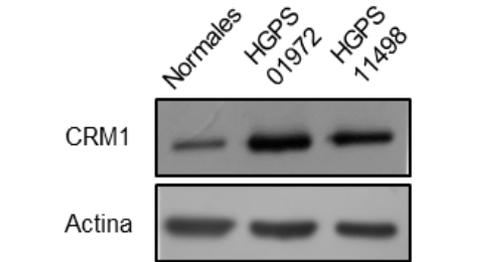


LA HIPERACTIVIDAD DE LA EXPORTINA CRM1 SE DEBE A SU SOBREEXPRESION EN LAS CELULAS HGPS

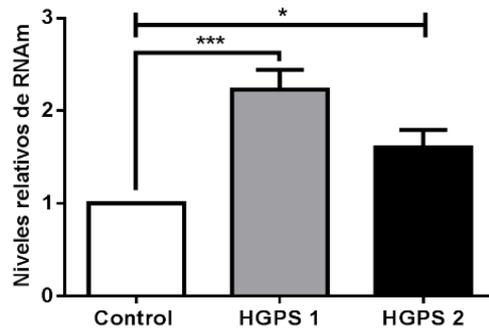
Localización subcelular de la exportina CRM1



Niveles proteicos de la exportina CRM1

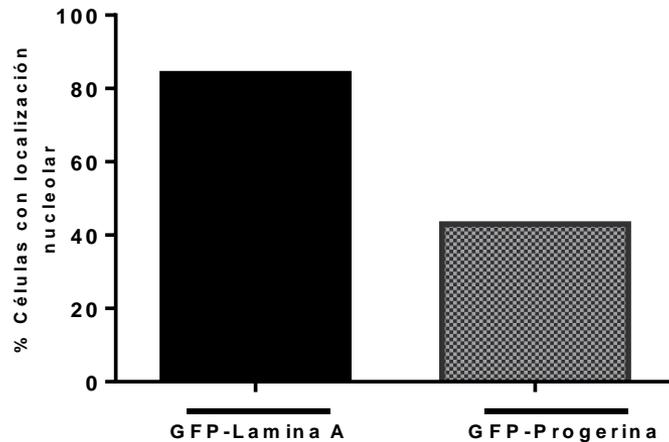
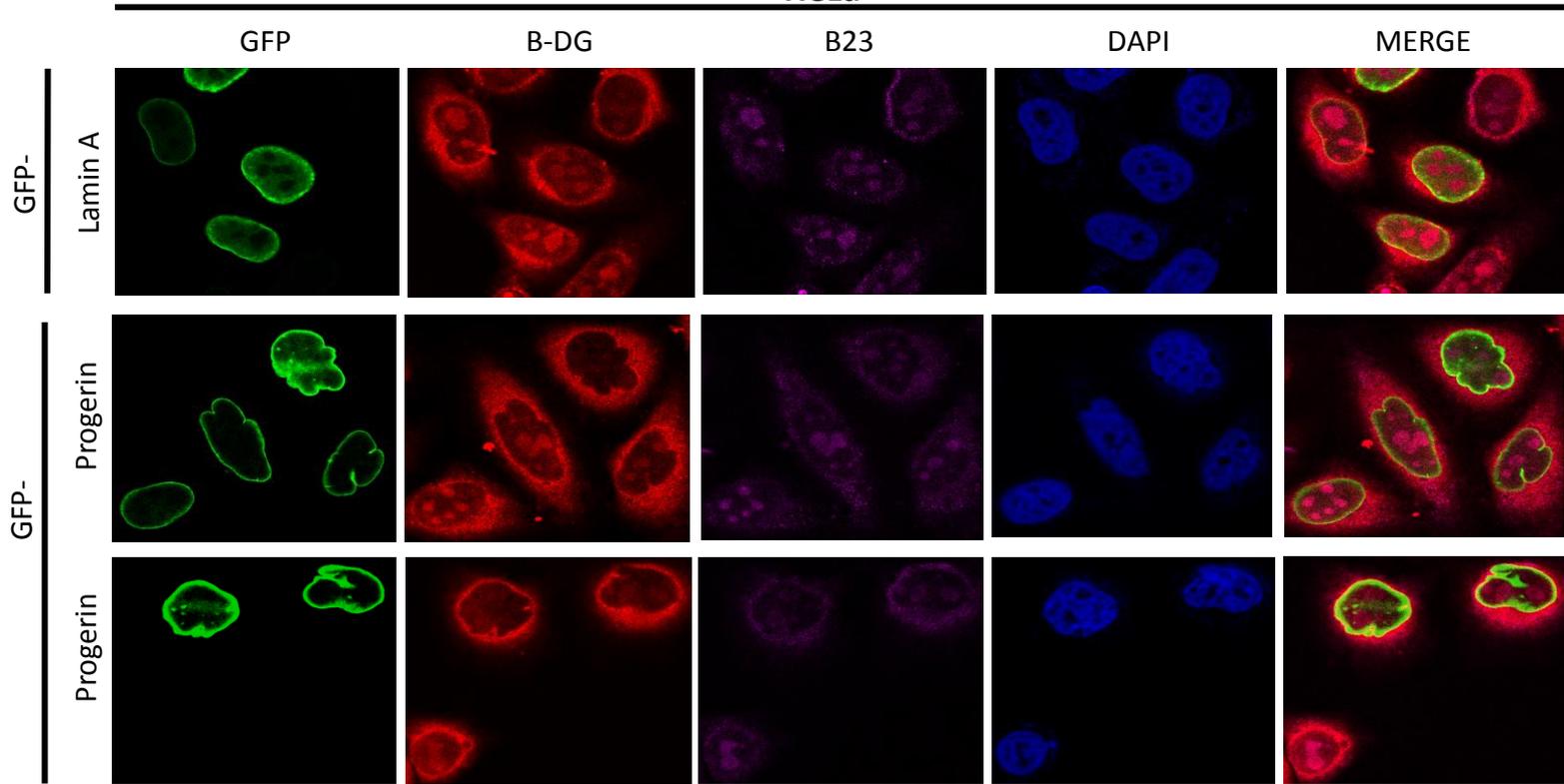


Niveles transcripcionales de la exportina CRM1

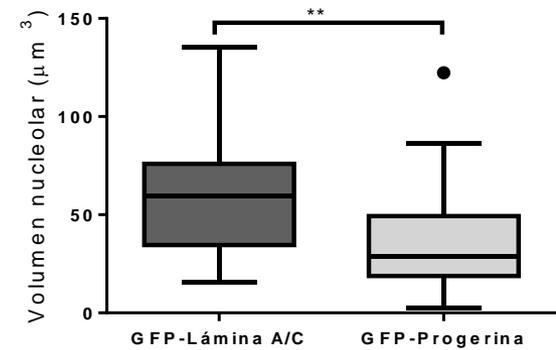


LA SOBRE-EXPRESION DE LA PROGERINA RECREA LA ALTERACIÓN DEL EXPORTE NUCLEAR EN LAS CELULAS HeLa

HeLa

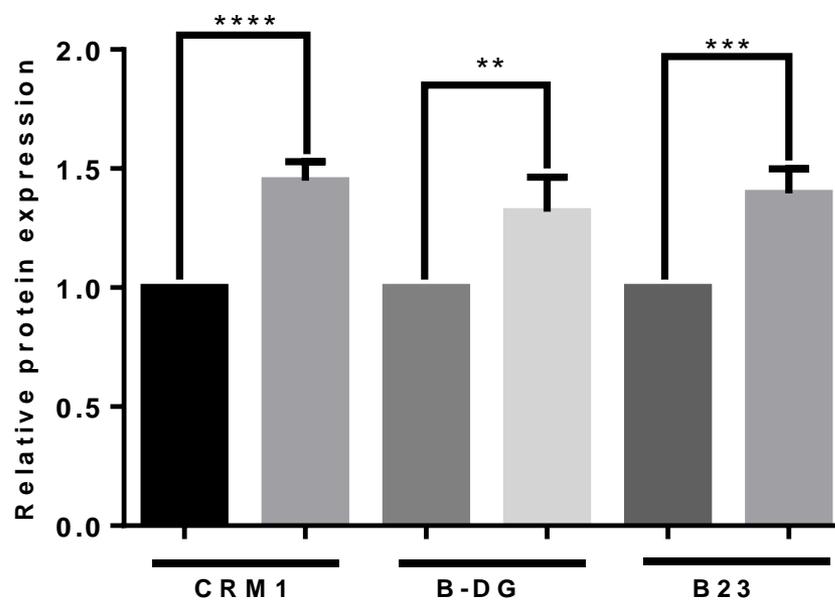
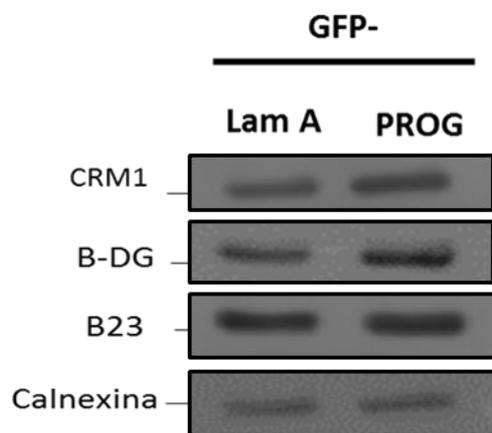


n=250



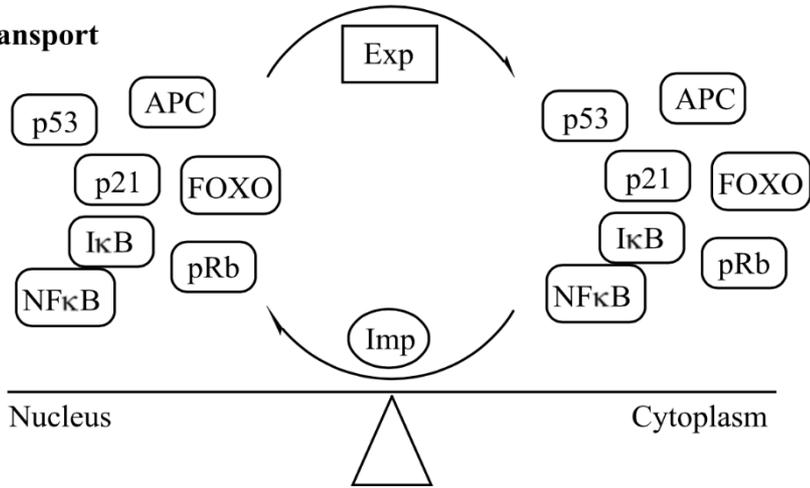
**P=0.005

LA SOBRE-EXPRESION DE LA PROGERINA INCREMENTA LOS NIVELES DE LA EXPORTINA CRM1 EN LAS CELULAS HeLa

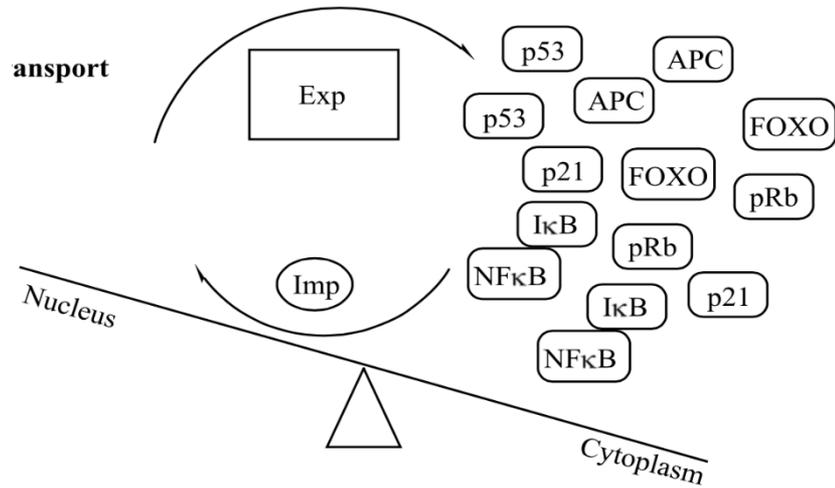


TRANSPORTE NUCLEAR EXACERBADO

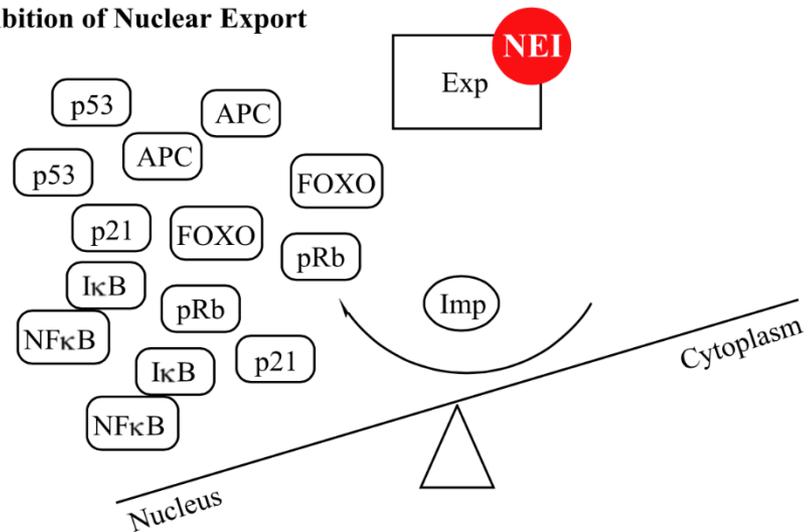
Transport



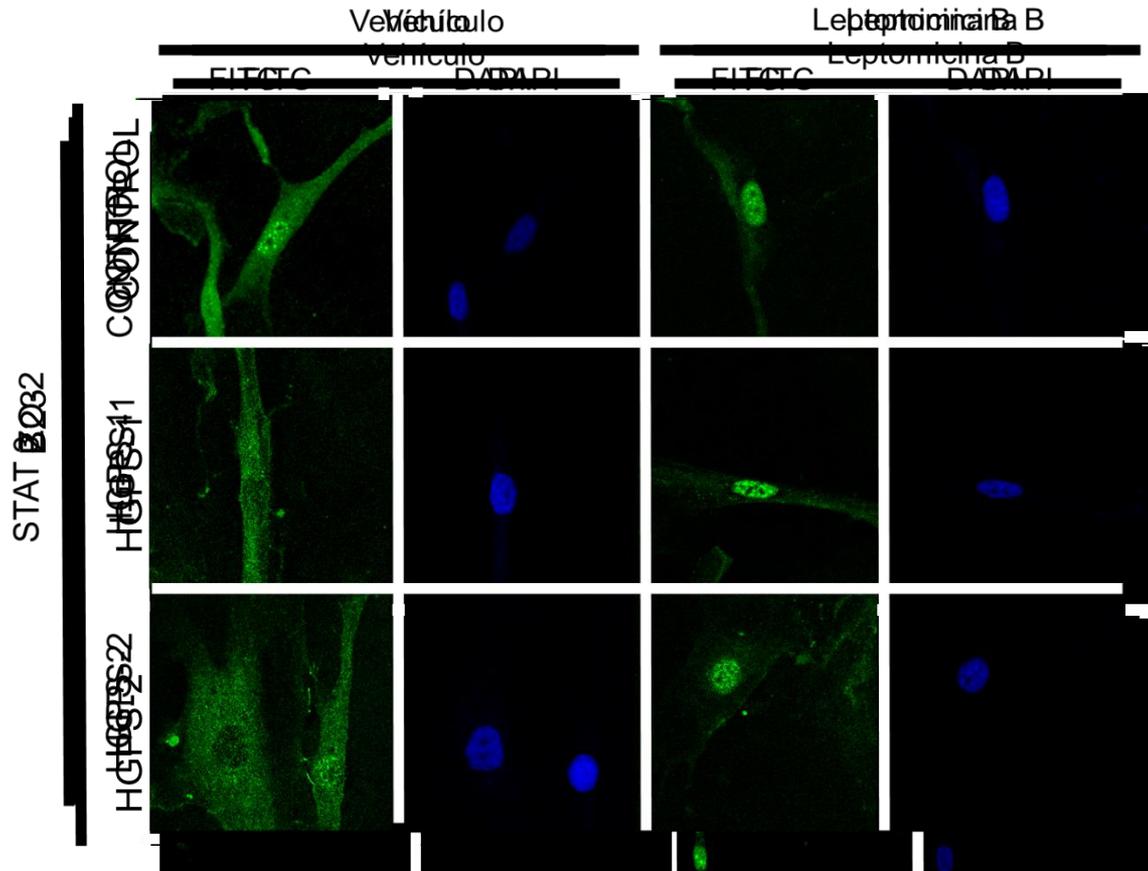
Transport



Inhibition of Nuclear Export



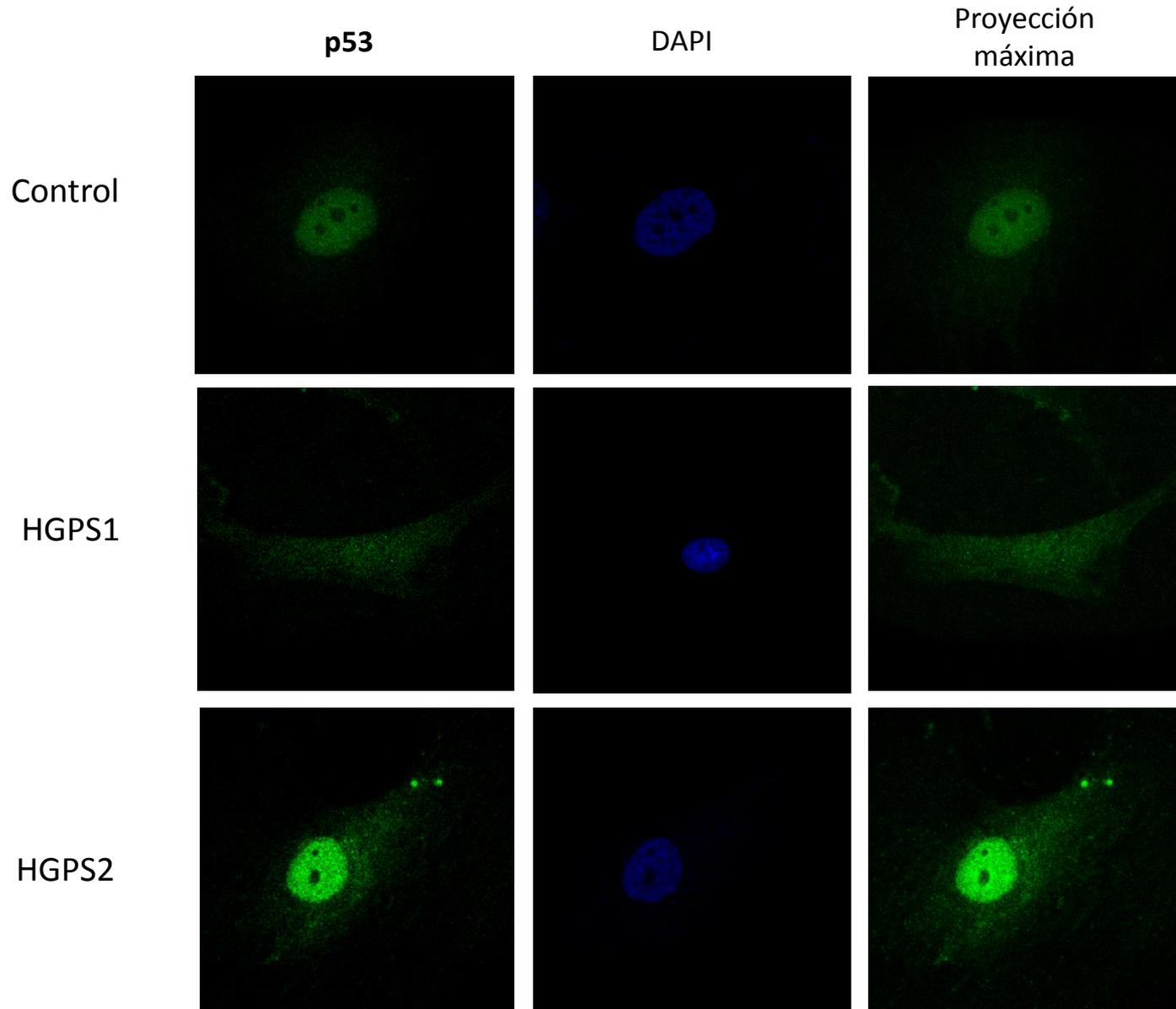
EL EXPORTE NUCLEAR EXACERBADO OCASIONA LA DISMINUCION DE LOS NIVELES NUCLEARES DE DIVERSAS PROTEINAS EN LAS LAS CELULAS HGPS



Consecuencias moleculares asociadas con la inhibición de CRM1

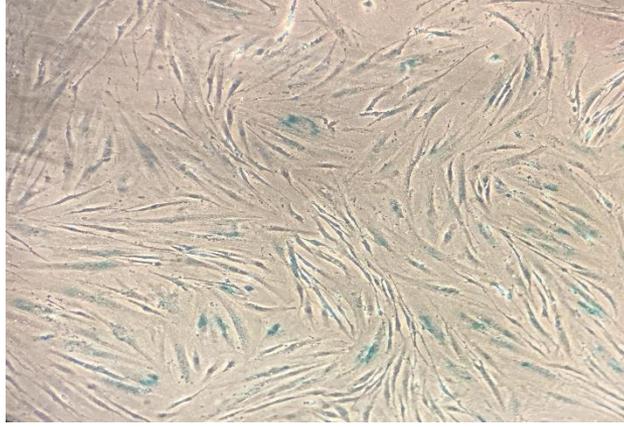
| Acumulación Nuclear | Efecto biológico | Referencias |
|---------------------|---|---|
| p53 | Restauración de p53 nuclear, respuesta al estrés mediada por p53, inhibición de la ruta de m-TOR, prevención de hipertrofia celular y morfología senescente. | Zoya N. et al., 2010; Giorgi C. et al., 2016. |
| B23 | Se <u>previene</u> su <u>interacción con proteínas pro-apoptóticas</u> , cuya interacción se lleva cabo por medio de la presencia de B23 en citoplasma, lo cual promueve apoptosis. | Thompson J. et al., 2008; Kerr L. et al., 2007; Khandelwal N. Et al., 2011. |
| ATF2 | Participación en el reclutamiento de proteínas relevantes en la reparación del daño al DNA. Se previene disfunción mitocondrial. | Bhoumik A. et al., 2005; Lau E. et al., 2012. |
| m-TOR | Biogénesis mitocondrial y autofagia. | Giorgi C. et al., 2016. |

Fibroblastos

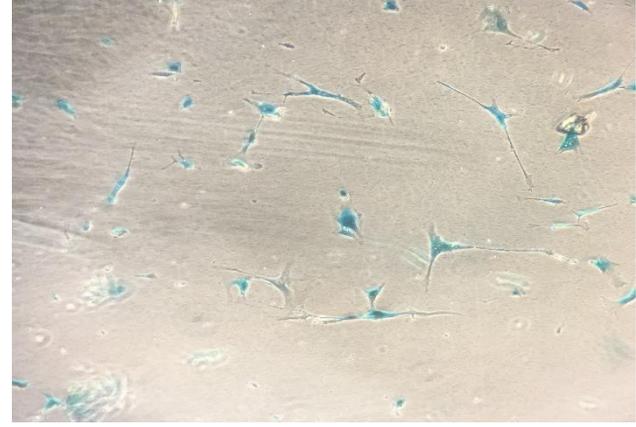


Etanol

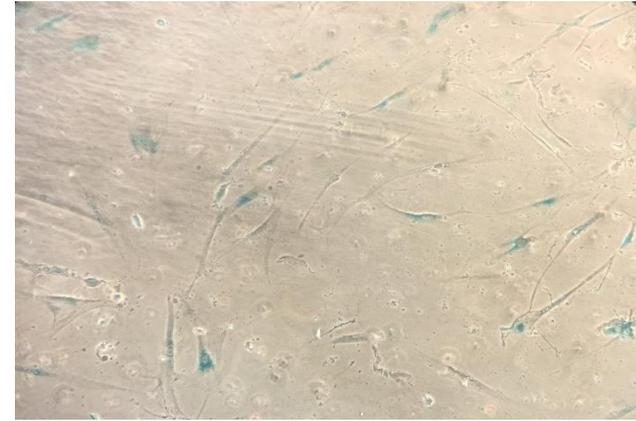
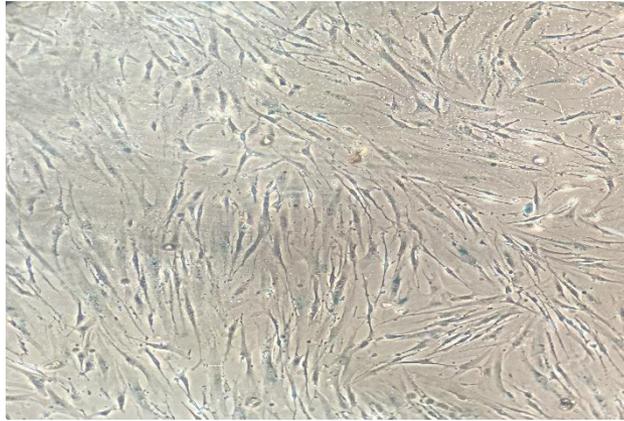
HGPS1



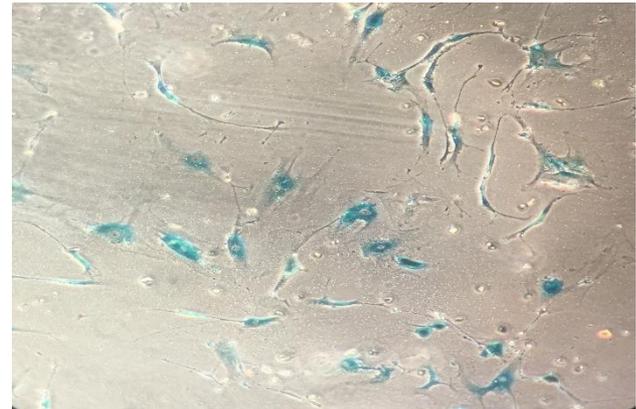
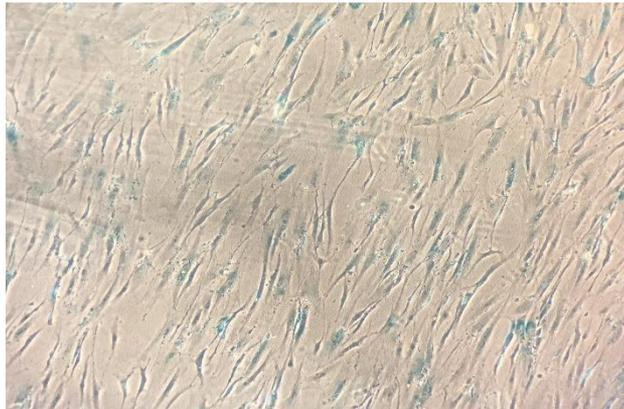
HGPS2



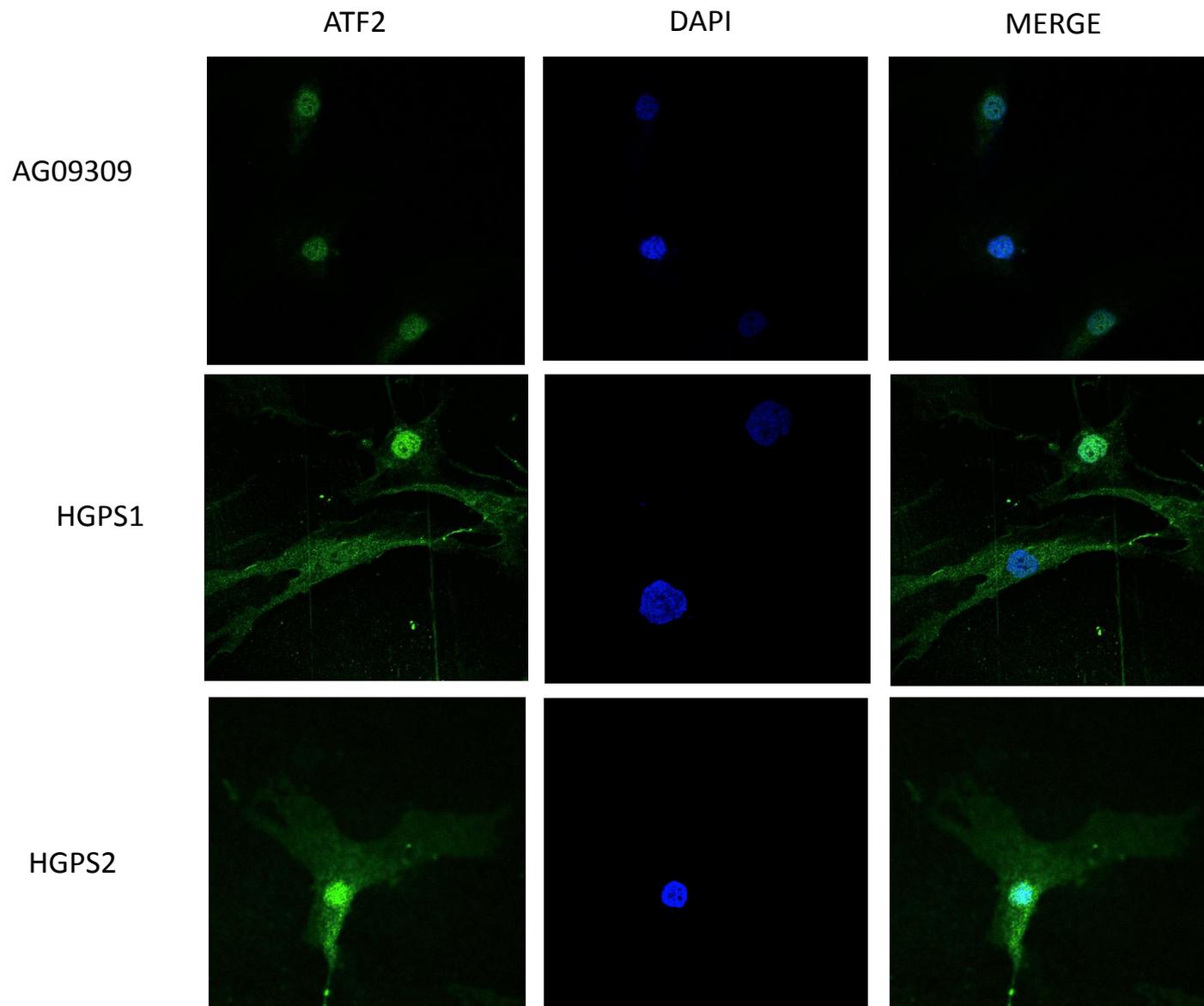
LMB



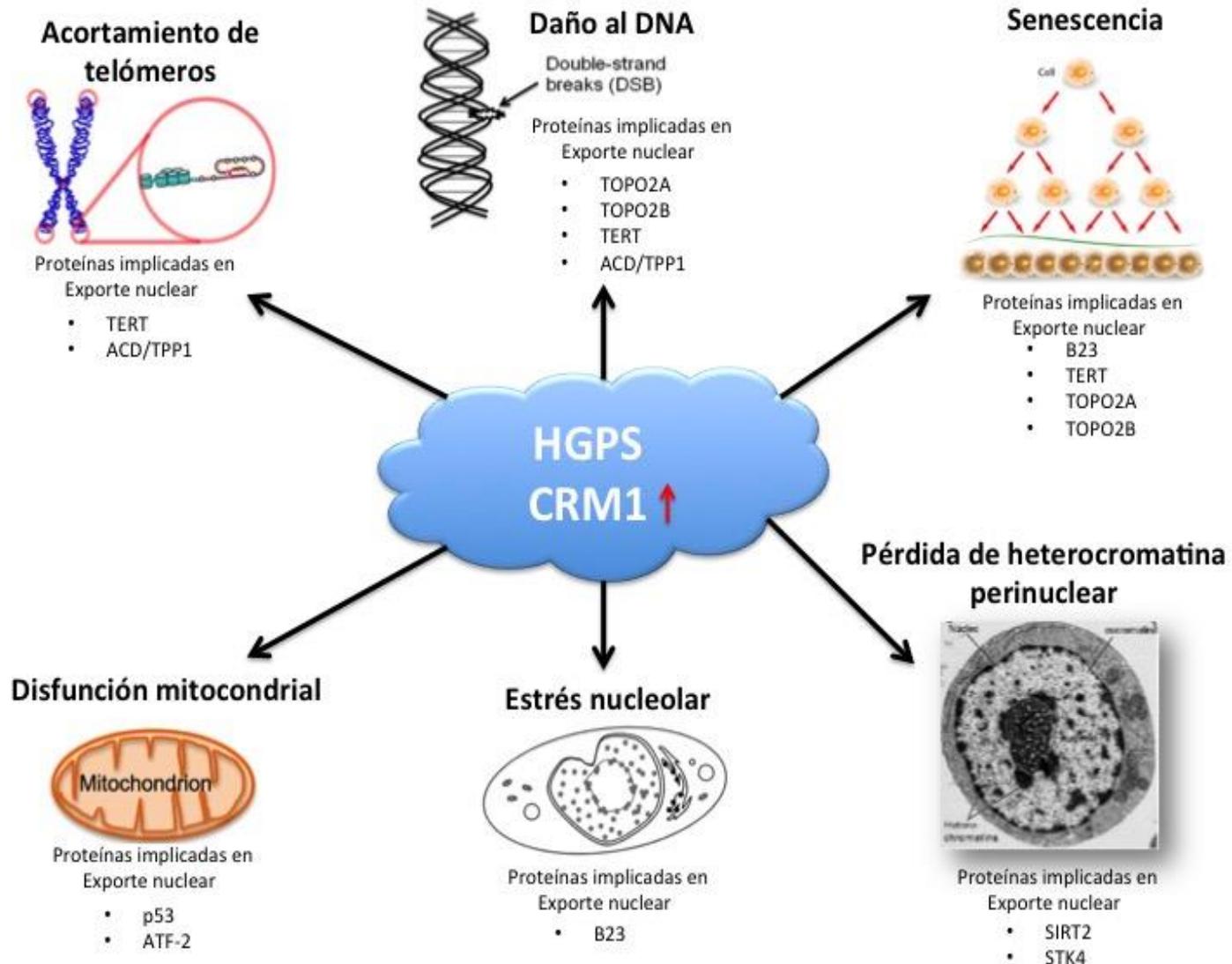
FTI



Fibroblastos

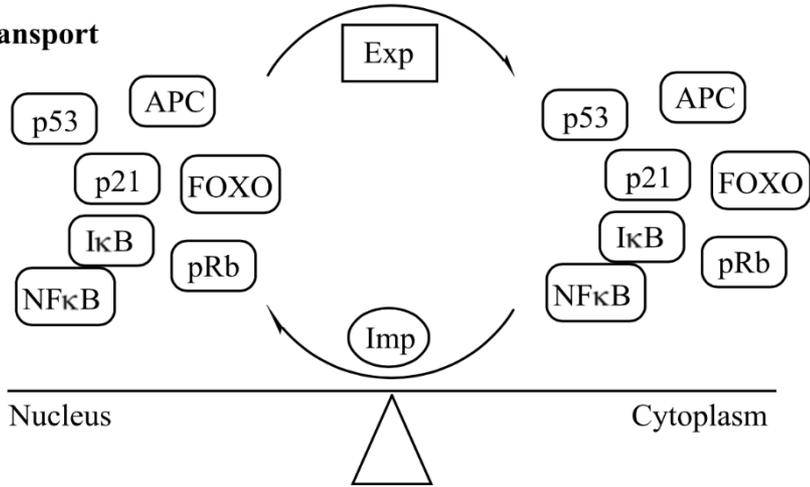


“POTENCIAL TERAPEUTICO DE LA MODULACIÓN DEL EXPORTE NUCLEAR”

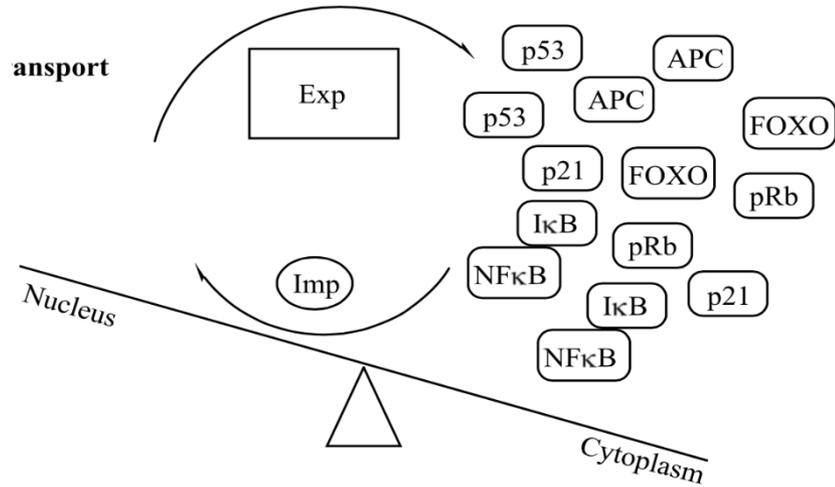


TRANSPORTE NUCLEAR EXACERBADO

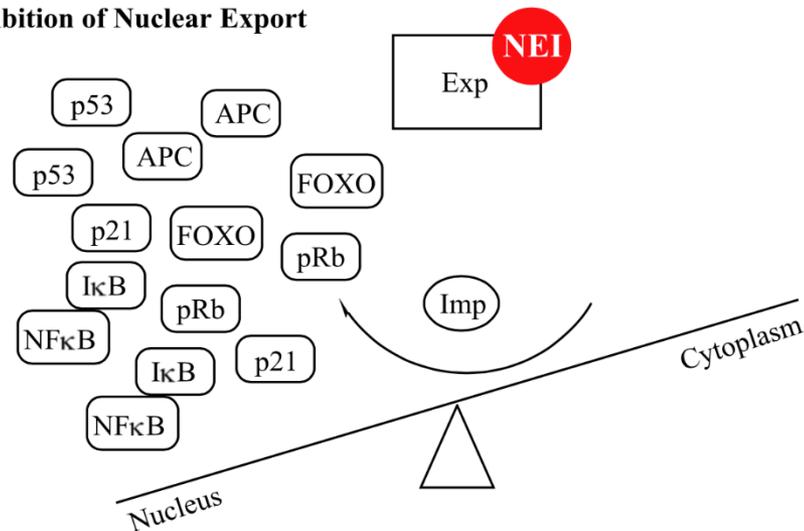
Transport



Transport

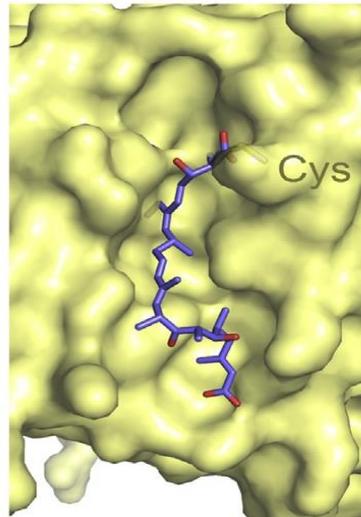


Inhibition of Nuclear Export

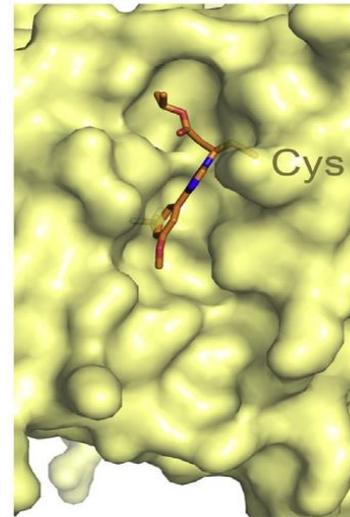


“Inhibidores selectivos del transporte nuclear (SINEs)”

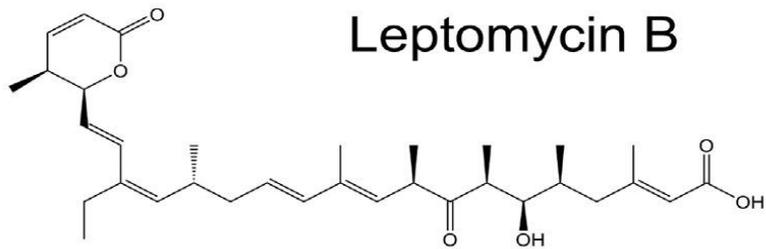
LMB



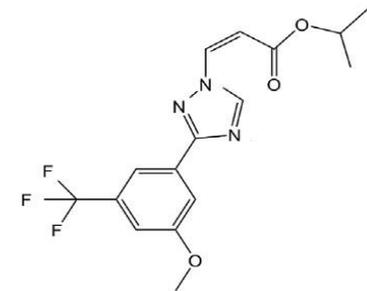
KPT-185



Leptomycin B



KPT-185



EVALUACION PRECLINICA DE LA TERAPIA MOLECULAR BASADA EN LA MODULACION DEL EXPORTE NUCLEAR

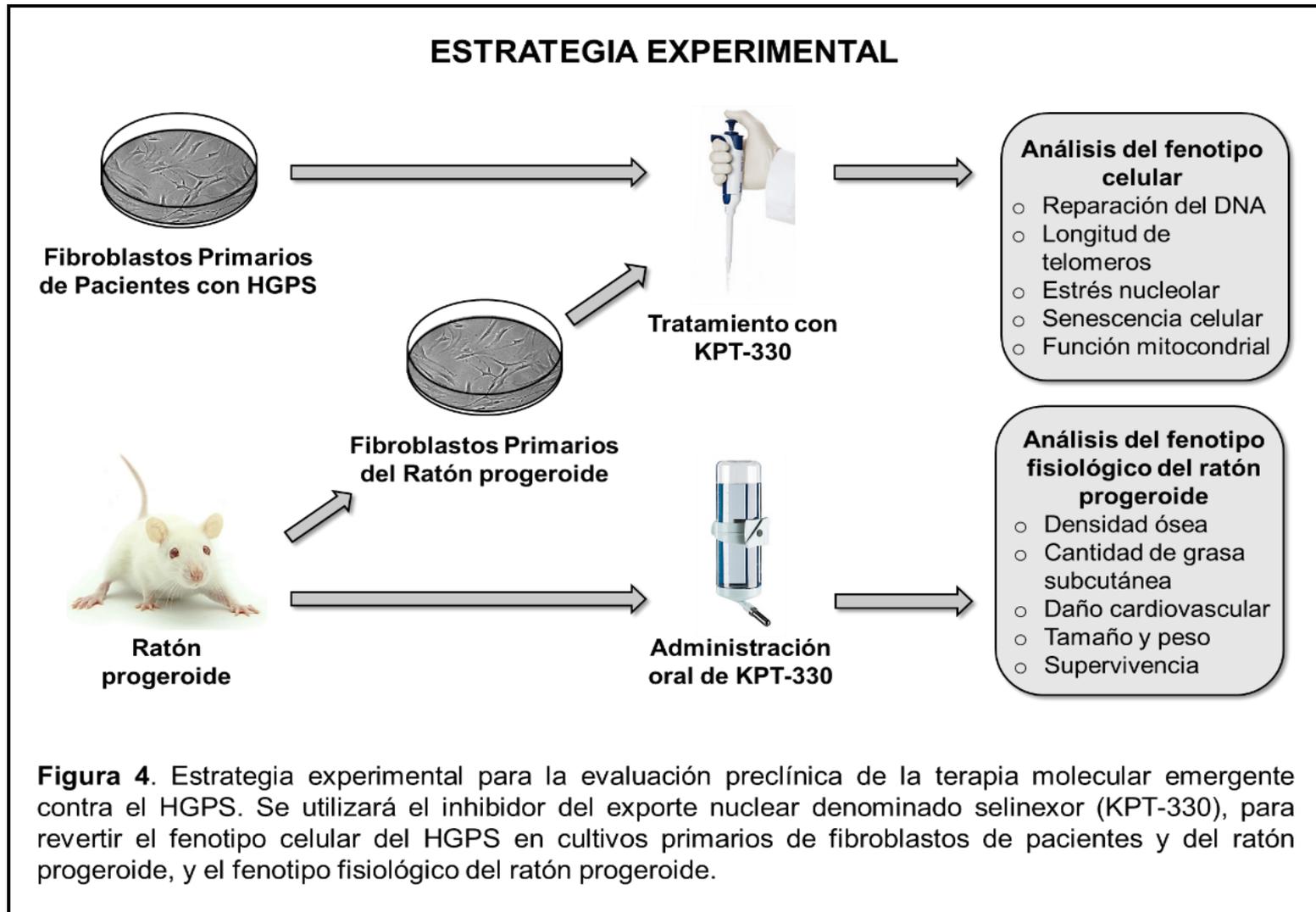


Figura 4. Estrategia experimental para la evaluación preclínica de la terapia molecular emergente contra el HGPS. Se utilizará el inhibidor del exporte nuclear denominado selinexor (KPT-330), para revertir el fenotipo celular del HGPS en cultivos primarios de fibroblastos de pacientes y del ratón progeroide, y el fenotipo fisiológico del ratón progeroide.

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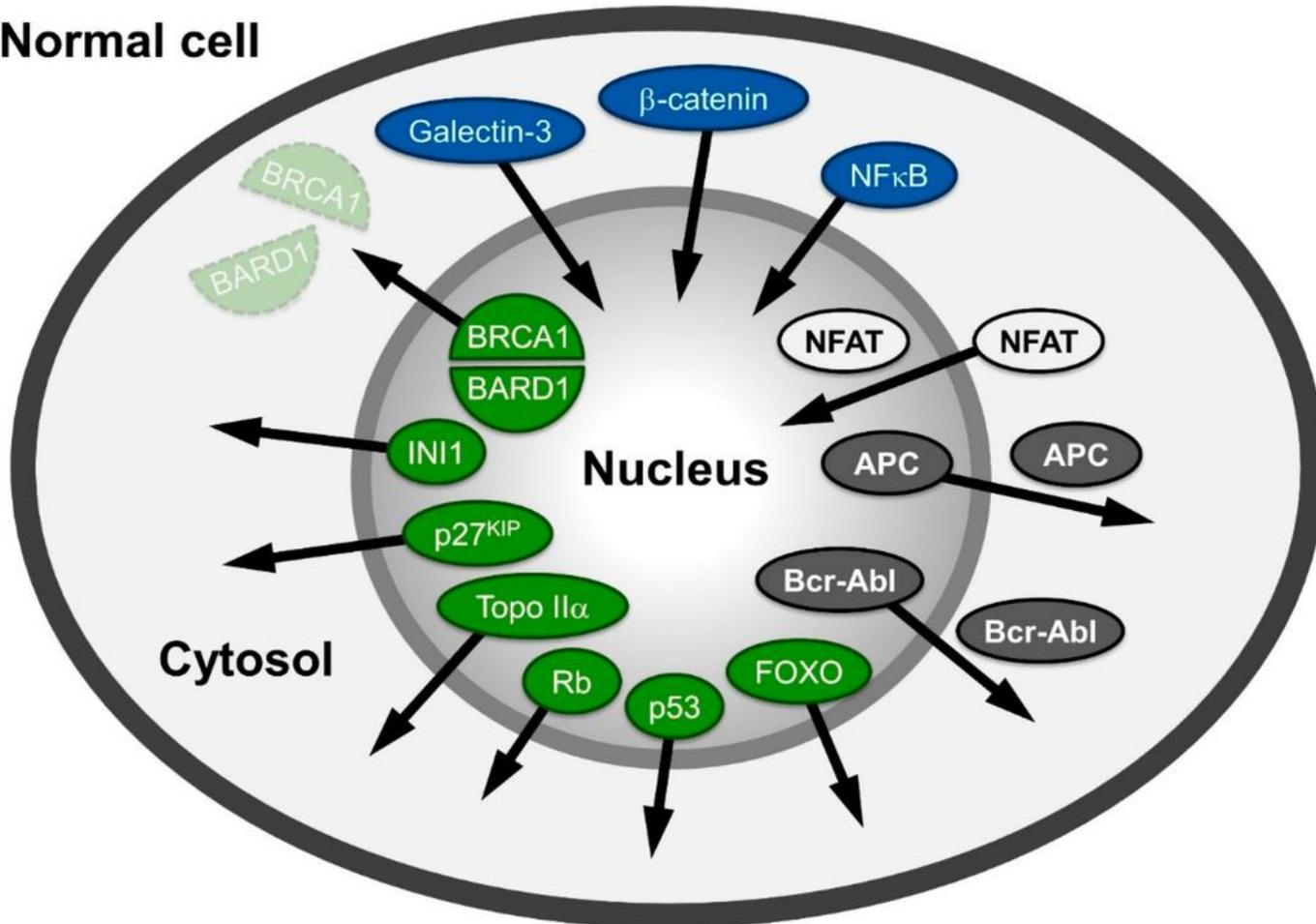
GRISELDA VELEZ

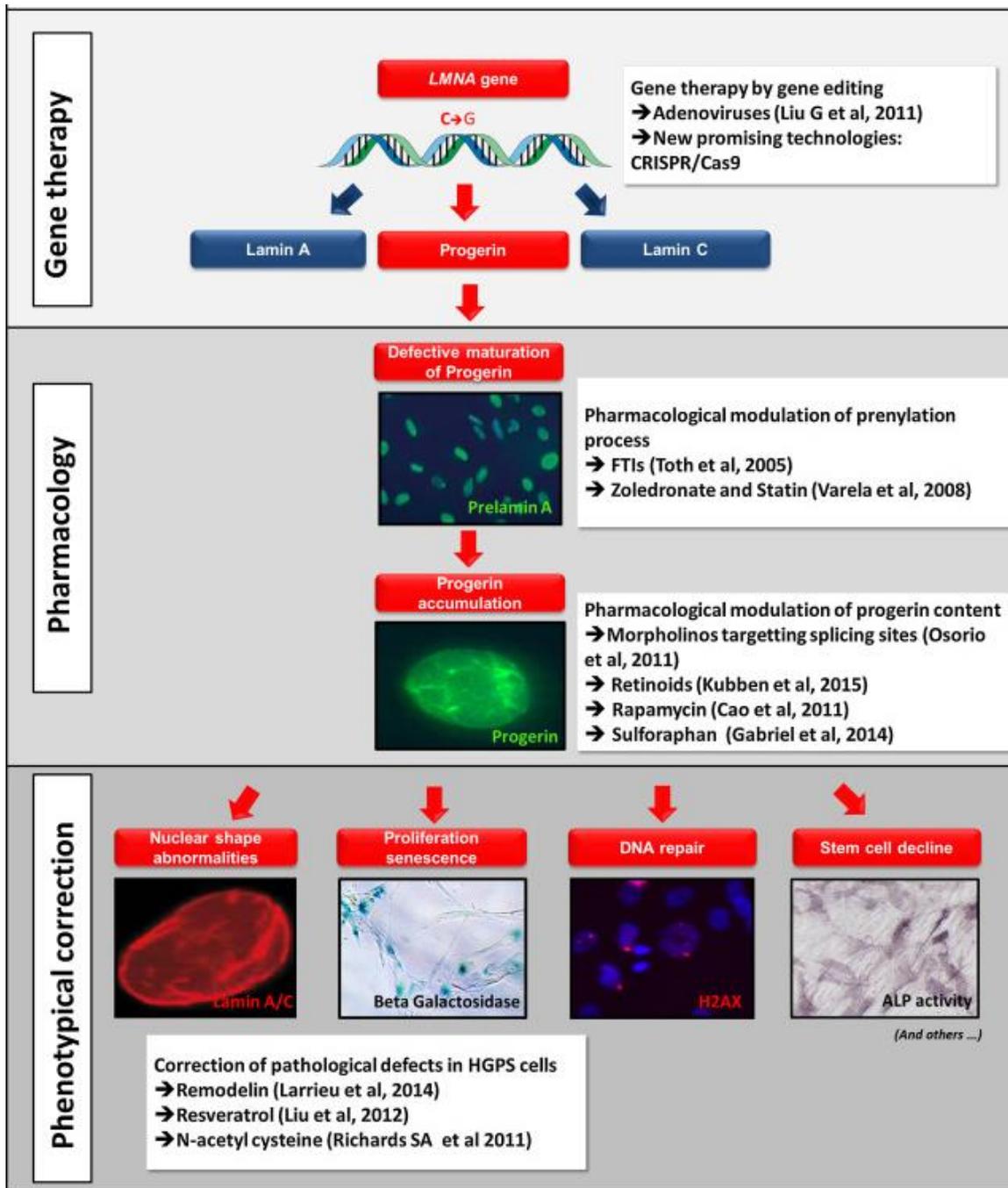
NELY ALAMILLO

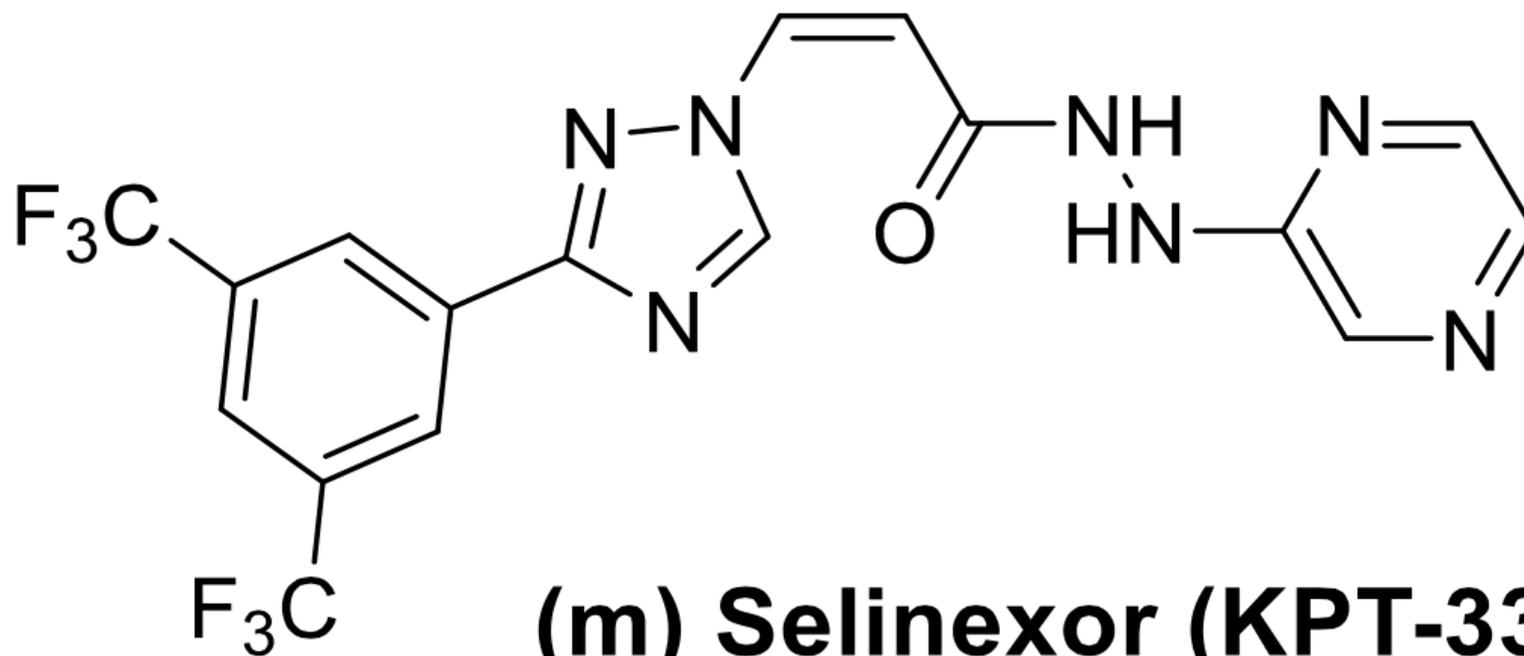


RELEVANCIA FISIOLÓGICA DEL EXPORTE NUCLEAR EXACERBADO

Normal cell

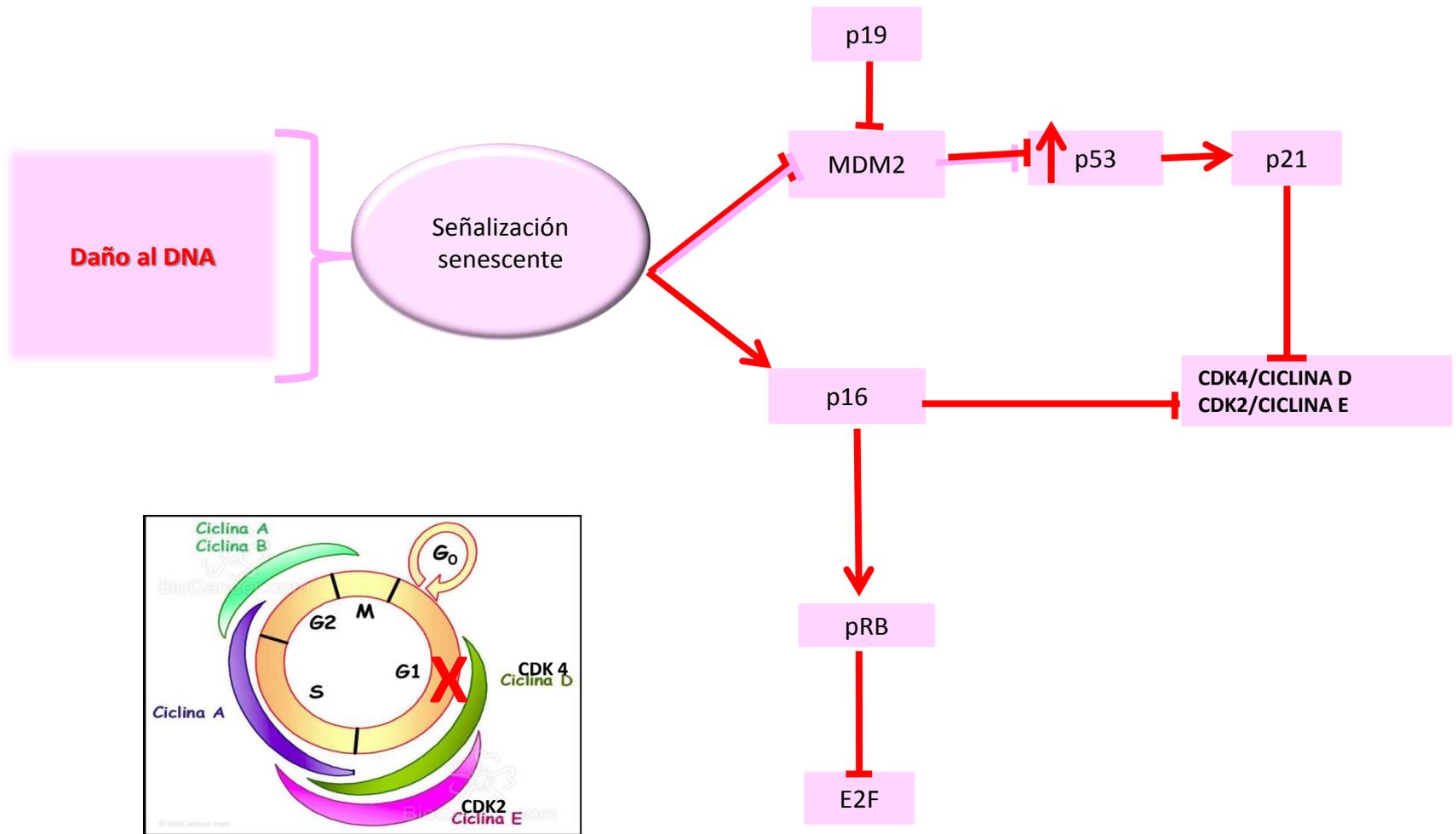




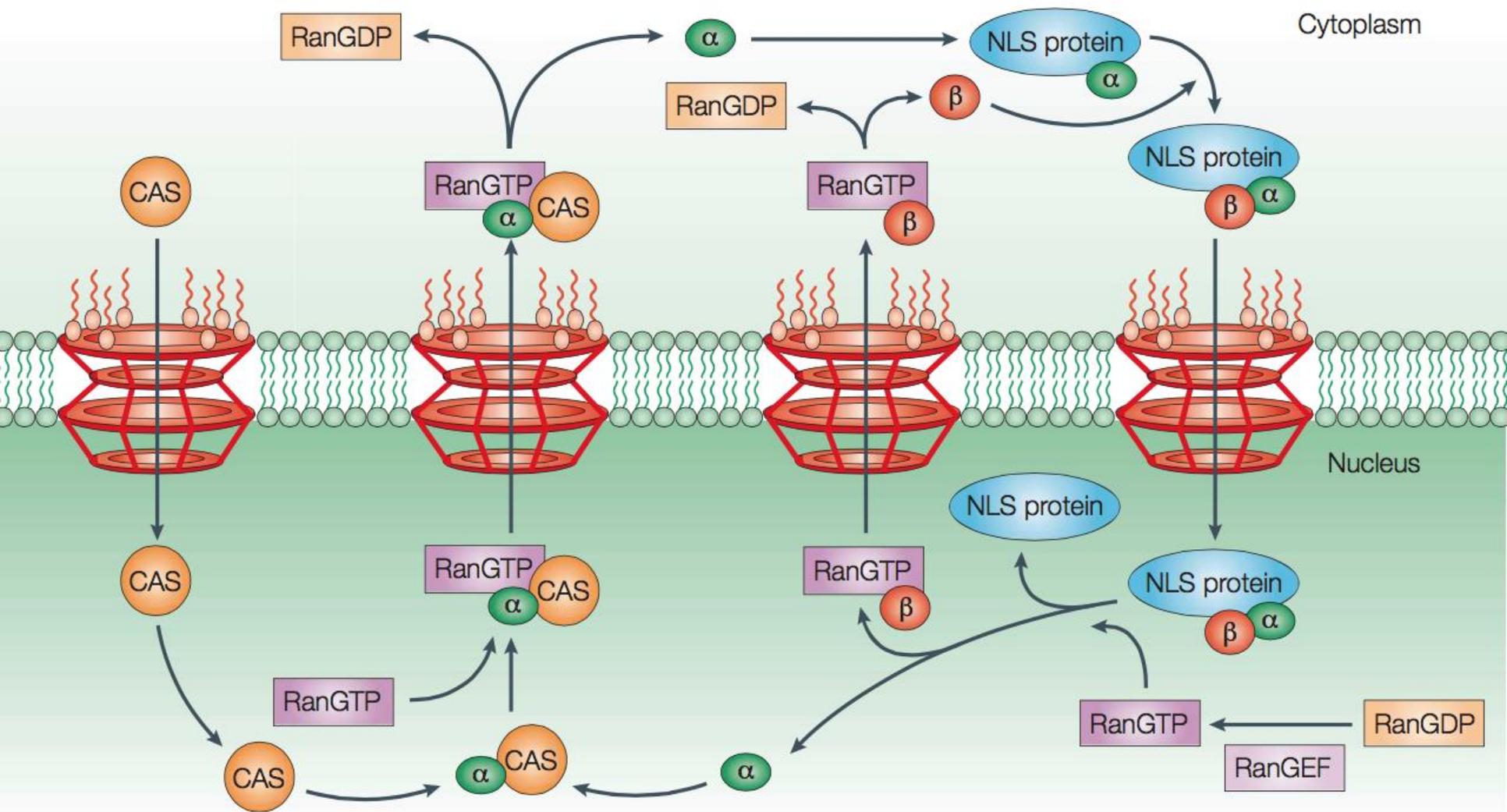


(m) Selinexor (KPT-330)

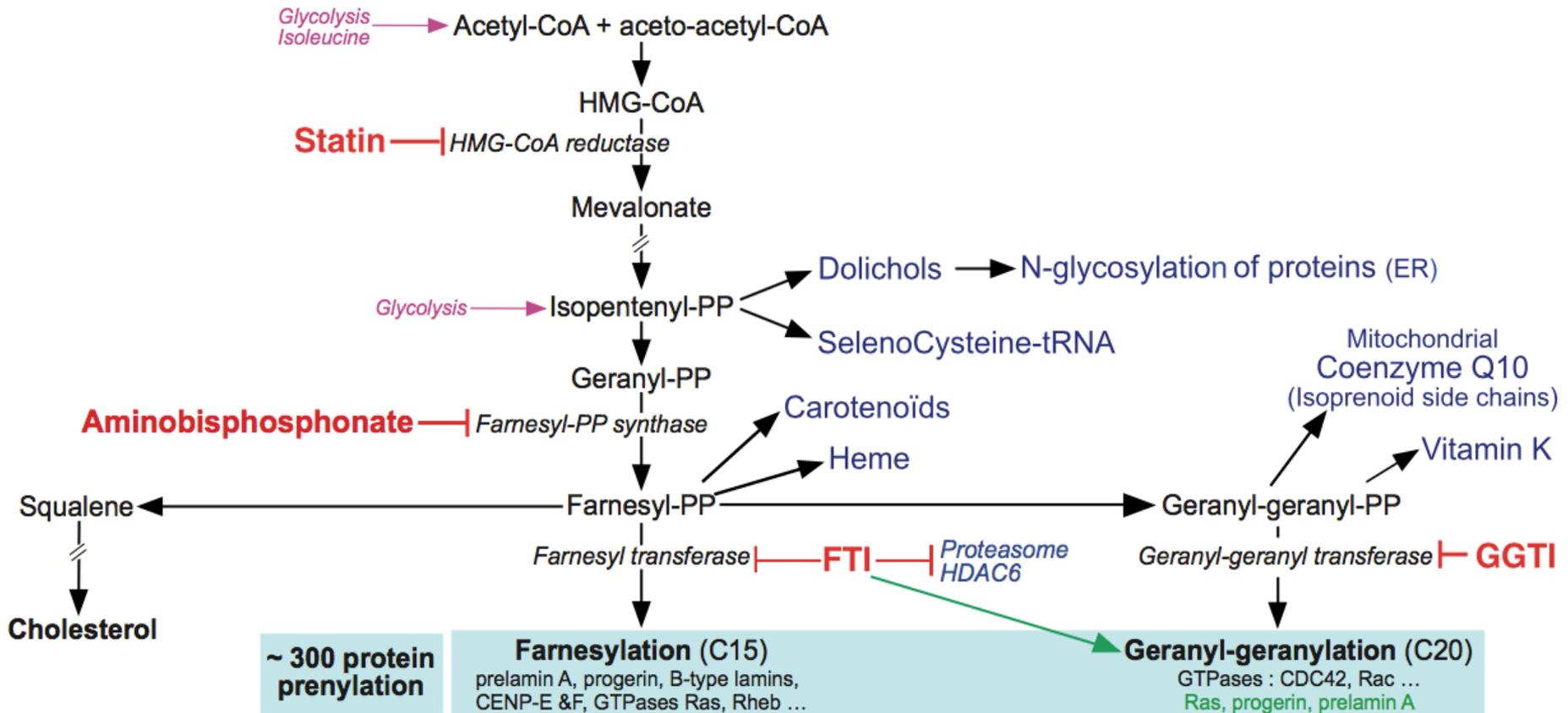
LA SENESCENCIA CELULAR Y EL SINDROME DE PROGERIA



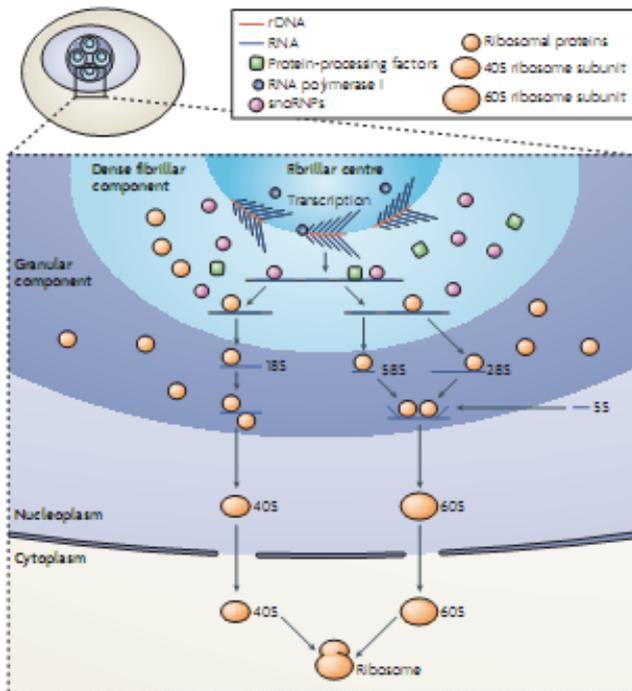
MDM2 (de sus siglas en inglés "murine double minute 2")



“Biosíntesis de isoprenoides”

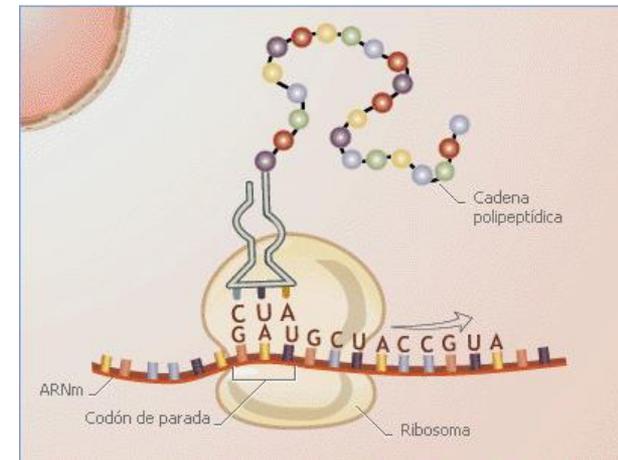


Biogénesis de los ribosomas

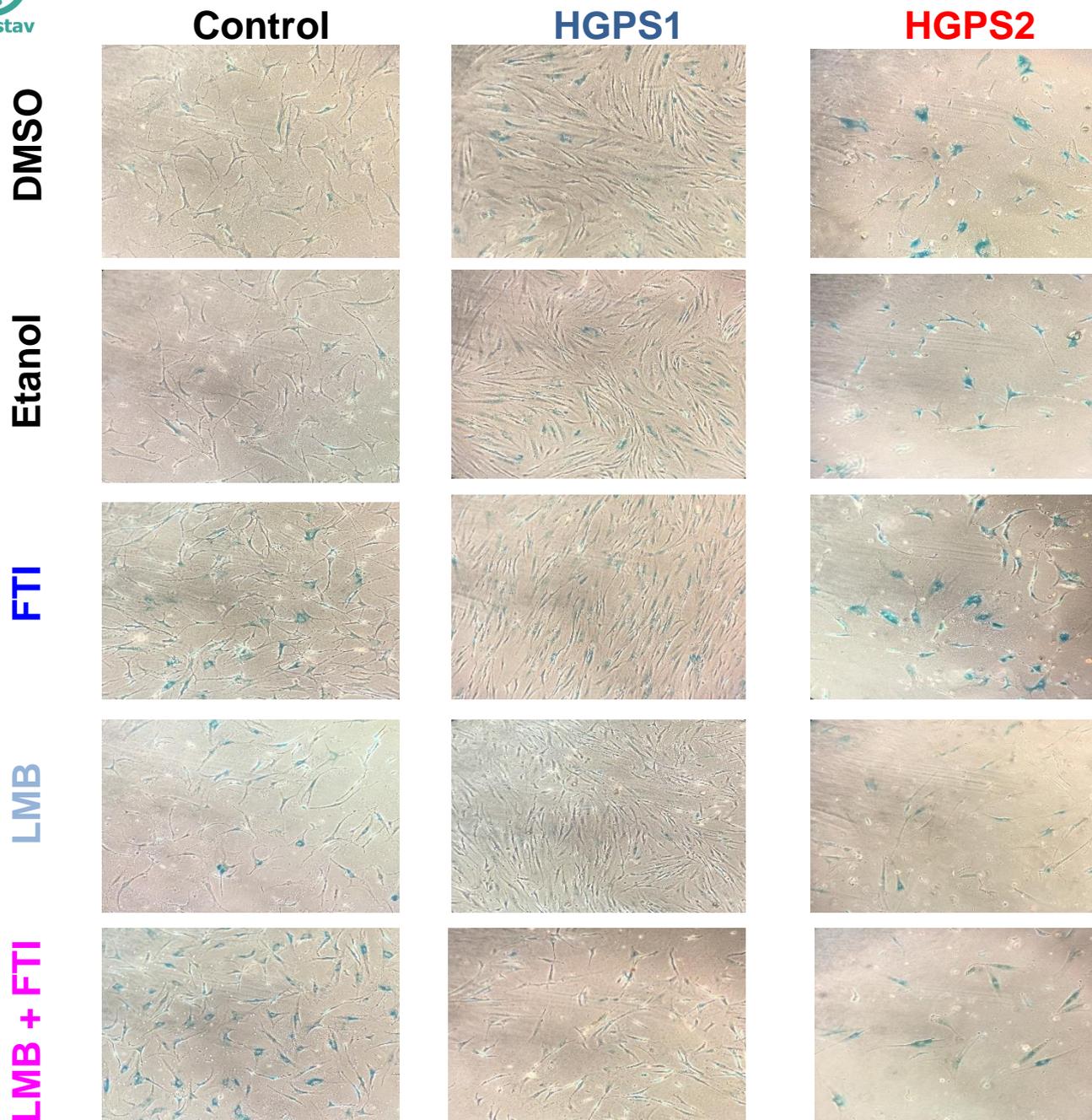


RNA polimerasa I:
transcribe rRNA

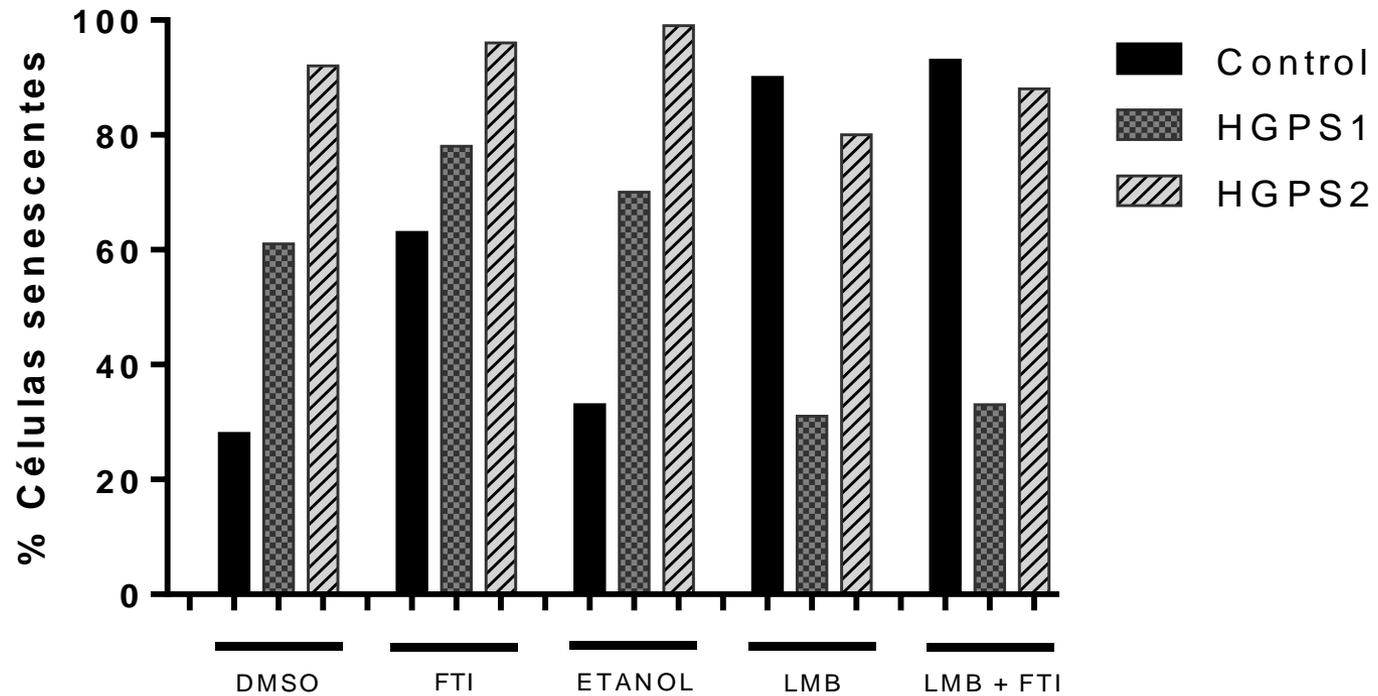
RNA polimerasa III:
síntesis de RNA
ribosomal 5s

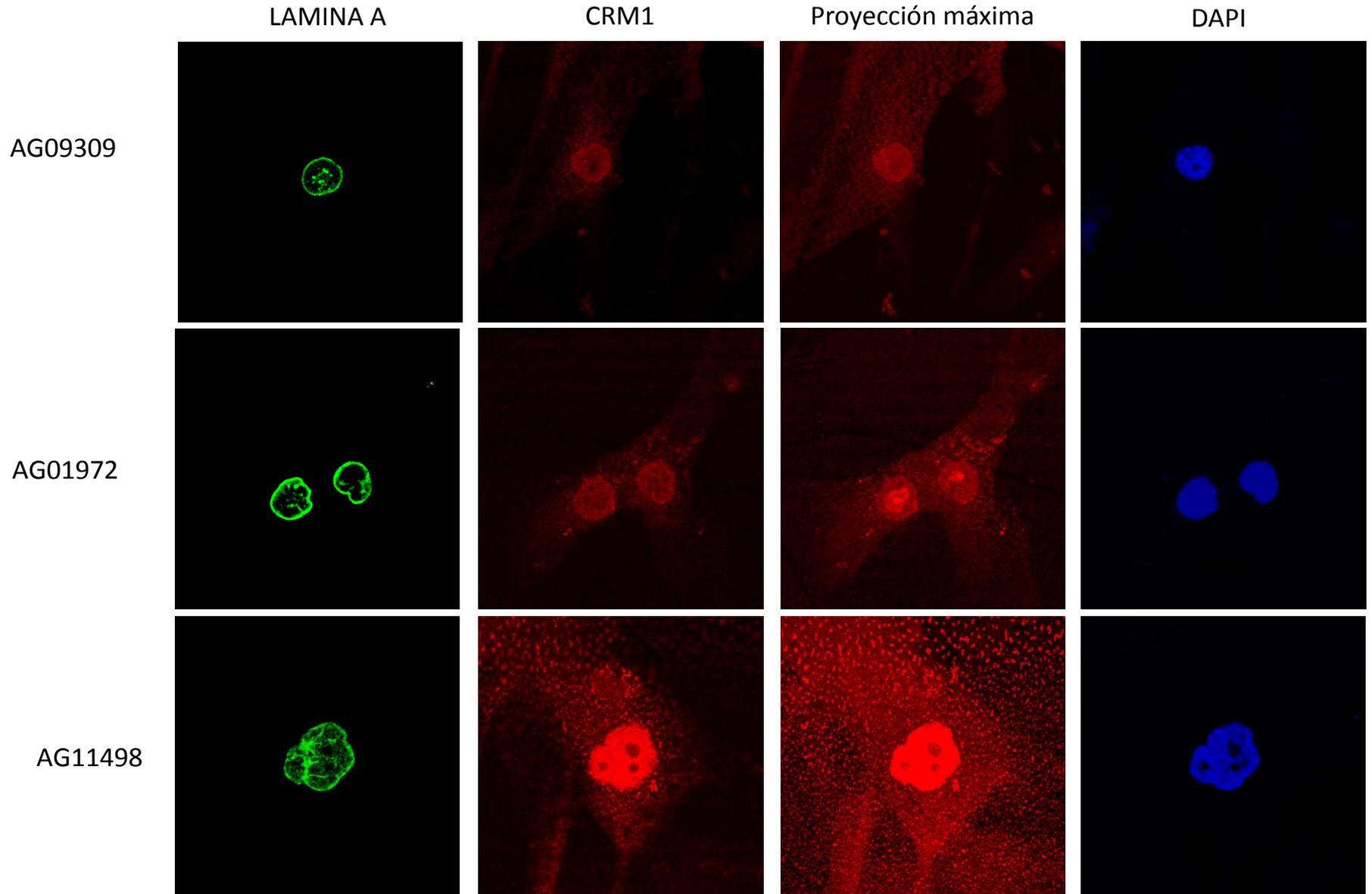


Fibroblastos



“Cuantificación”





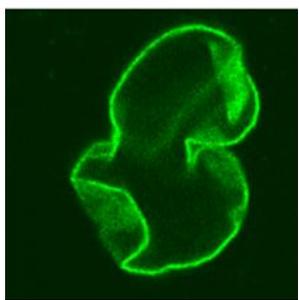
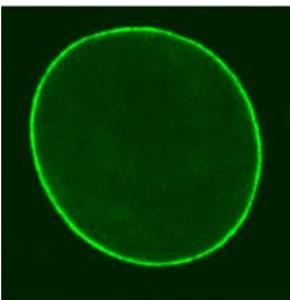
MARCAS DISTINTIVAS DE LAS CÉLULAS DE PROGERIA

Inmunofluorescencia de la lámina A/C

Núcleo de una célula de un individuo sano

Núcleo de una célula de un paciente con progeria

WT



MUT

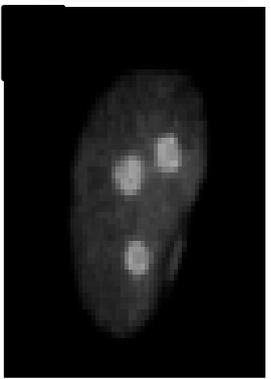
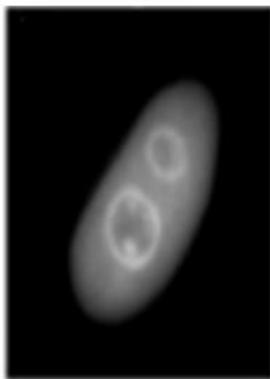
Scaffidi P, Gordon L, Misteli T (2005)

Control

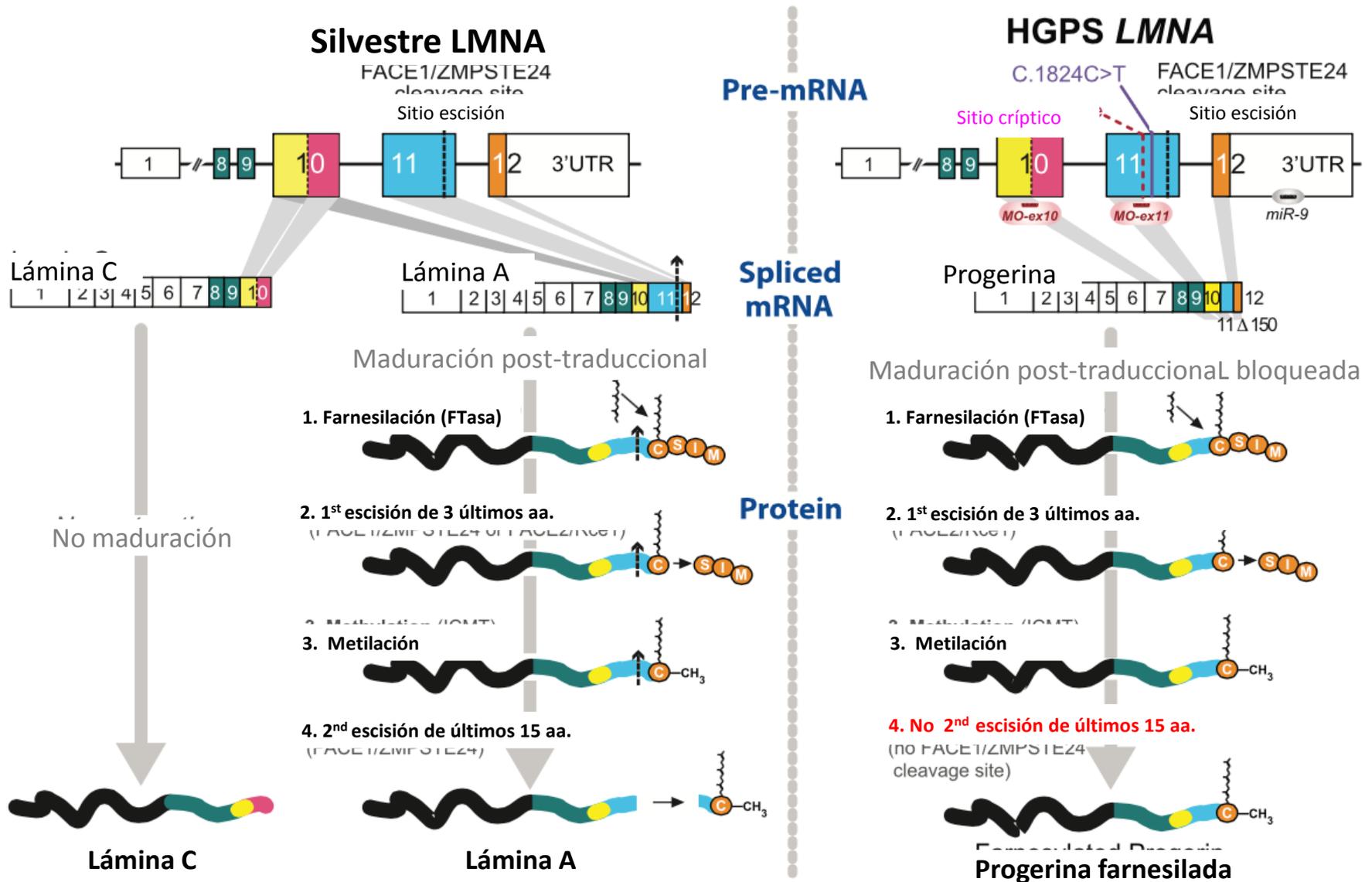
Célula HGPS

HGPS + FTI

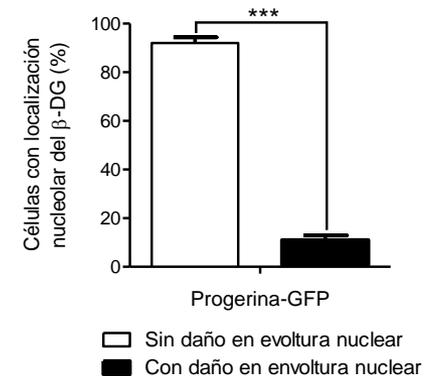
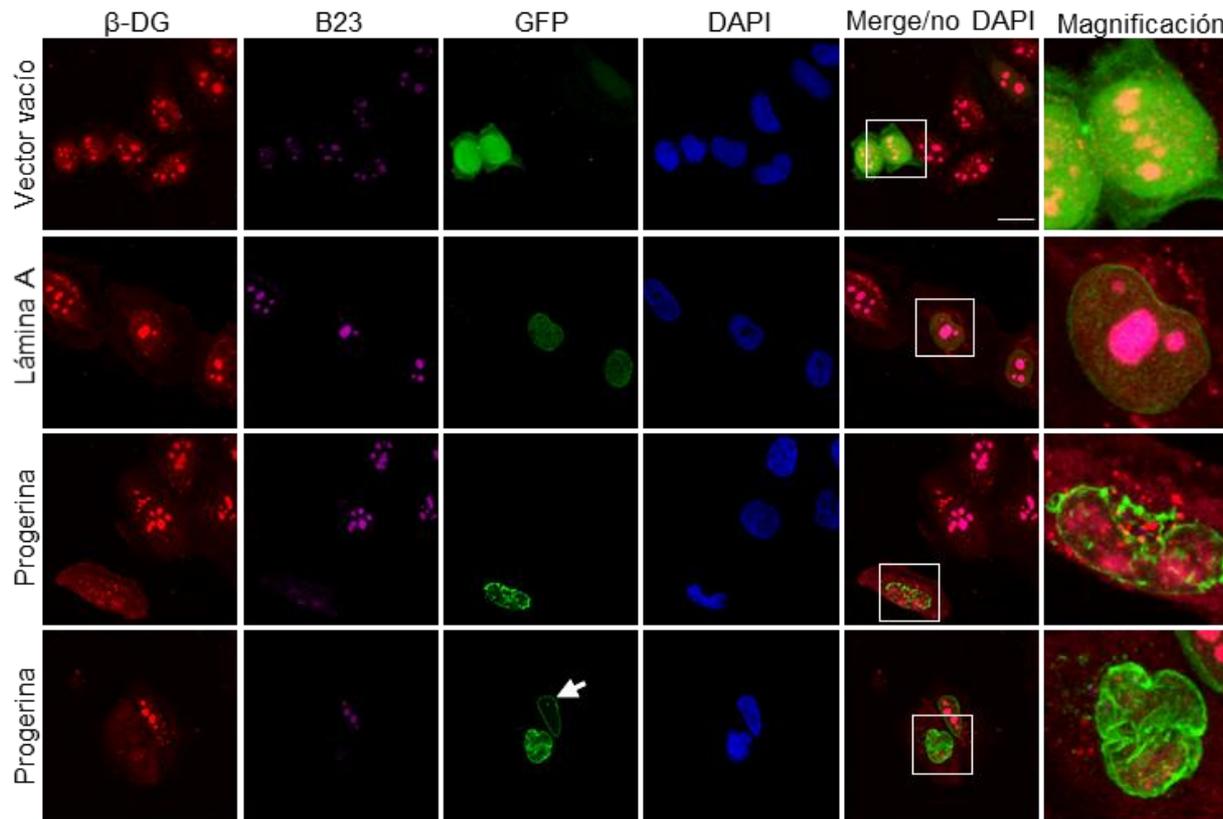
Nucleolina



“Biosíntesis y maduración de lámina A, C y Progerina”



LA SOBRE-EXPRESION DE LA PROGERINA RECREA LA ALTERACIÓN DEL EXPORTE NUCLEAR EN LAS CELULAS HeLa



Dermopatía restrictiva (RD) – pre-lámina A



- Retraso del crecimiento intrauterino
- Boca abierta
- Micrognacia
- Piel translúcida y delgada
- Lesiones esclerodermiformes
- Hipoplasia pulmonar
- Osteolisis de las falanges distales

ZMPSTE24

Dos mutaciones nulas

OMIM 275210

[141] [142]

Síndrome de Progeria atípico (APS)



- Retraso en el crecimiento
- Proptosis
- Micrognacia
- Apiñamiento dental
- Alopecia
- Osteolisis de las falanges distales
- Osteopenia
- Lipodistrofia

LMNA

c.1583C>T, p.T528M
and
c.1619T>C, p.M540T

[180]

Síndrome de Progeria de Nestor-Guillermo (NHPS)



- Retraso en el crecimiento
- Lipoatrofia generalizada
- Piel seca atrófica con defectos de pigmentación
- Apiñamiento dental
- Osteoporosis
- Deformaciones esqueléticas

BANF1

c.34G>A, p.A12T
Homocigoto

OMIM 614008

[143] [181]

Síndrome de Hallermann-Streiff (HSS)



- Braquicefalia con abombamiento frontal
- Hipotricosis
- Cataratas y microftalmia
- Micrognacia
- Anomalías dentales
- Corta estatura

Gen desconocido

OMIM 234100

Hennekam R.C.M, courtesy

LAMINOPATÍAS

